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# Spectroscopic and chromatographic determination of fluvastatin sodium in presence of its acid degradate

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**Abstract:** Six new selective and precise methods were developed and validated for the determination of fluvastatin sodium (Flu) in pure form, in presence of its acid degradate and in pharmaceutical formulations. The degradate was isolated, via acid degradation, characterized and confirmed. The first method was a third derivative ($^3$D) method which allows the determination of Flu at 318.6 nm (zero-crossing of its acid degradate) over the concentration range of 4 – 20 μg.mL$^{-1}$ for Flu. The second and third methods were first and second derivative ratio techniques ($^1$DD and $^2$DD). The measurements were taken at 240.4 nm ($^1$DD$_{240.4}$), 259.4 nm ($^1$DD$_{259.4}$), 294.8 nm ($^1$DD$_{294.8}$) ($^1$DD method), at 250.4 nm ($^2$DD$_{250.4}$) and at 264.2 nm ($^2$DD$_{264.2}$) ($^2$DD method) over the concentration range 4 – 20 μg.mL$^{-1}$, for Flu, ($^1$DD and $^2$DD methods), using normalized spectra as divisors. The fourth method was a sensitive spectrofluorimetric method which was based on measuring the native fluorescence intensity of FLU in ethanol at 776 nm with excitation at 258 nm, over the concentration range of 1 – 10 μg.mL$^{-1}$. The fifth method was based on separation of Flu from its acid degradate followed by densitometric measurements of the spots at 304 nm. The separation was carried out on silica gel F$_{254}$ plates using chloroform : hexane : methanol : glacial acetic acid   (5 : 5 : 1 : 1, v/v/v/v) as developing system. This method allows the determination of FLU over a concentration range of 1 – 10 μg/spot with mean percentage recovery 100.07 ± 0.935. The sixth method was based on high performance liquid chromatographic (HPLC) separation of Flu from  its acid degradate on reversed phase Zorbax C$_{18}$ column, using methanol : water (80 : 20, v/v) as mobile phase at a flow rate of 1 mL.min$^{-1}$ and sodium benzoate was used as internal standard (IS) with UV detection at 242 nm. Linear relationship was obtained over the concentration range of  5 – 50 μg.mL$^{-1}$. The selectivity of the proposed methods was checked using laboratory prepared mixtures and satisfactory results were obtained. The proposed methods have been successfully applied to the analysis of FLU in pharmaceutical dosage form and the validity of these methods was ascertained by applying the standard addition technique. The results were statistically compared with the reported USP method and no significant difference was found with respect to both precision and accuracy. Five of the suggested methods have the advantage of being stability indicating. Therefore, they can be used for routine analysis of the drug in quality control laboratories.

**Keywords :** Antihyperlipidemic; Densitometry; Fluvastatin sodium; RP-HPLC; Stability ; Third derivative and derivative ratio spectrophotometry.

## 1. Introduction

Fluvastatin sodium (6-Heptenoic acid, 7-[3-(4-fluorophenyl)-1-(1-methylethyl)-1_H_-indol-2-yl]-3,5-dihydroxy-monosodium salt, [_R*_,_S*_-(E)]- (±)-), Fig.1., is the first–generation synthetic statin, used as antihyperlipidemic drug[1]. It is a potent inhibitor of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, the rate limiting enzyme in cholesterol biosynthesis[2,3]. Flu is an official drug according to USP[1].

Different analytical techniques were reported for the determination of FLU in pharmaceutical dosage form and in biological fluids. These methods include voltammetric[4,5], UV spectrophotometric[6], gas chromatographic[7,8], capillary electrophoresis[9] and HPLC[10-14] methods. In addition, enantioselective analysis of FLU in plasma was carried out using liquid chromatographic–electrospray tandem mass spectrometric method[15,16].

The International conference on harmonization of technical requirements for registration of pharmaceuticals for human use (ICH) guideline states that the degradation products formed under a variety of conditions should be identified and degradation pathways established[17,18]. Furthermore, according to current good manufacturing practices, all drugs must be tested with a stability indicating assay method before release[19]. From the above considerations, it is clear that the investigation of drug stability represents an important issue in drug quality evaluation.

All recent publications concerning FLU lack data about its potential degradation products and hence, none of the methods are mentioned to be stability indicating. Consequently, it was thought necessary to develop simple, accurate, sensitive and stability indicating methods for the analysis of Flu in pure form and in presence of its degradate without the need of prior separation step. In addition, application of the proposed procedures to the analysis of the available pharmaceutical dosage form with satisfactory precision was also an important task for good analytical practice.

In the present investigation, stability study of FLU under acidic conditions was carried out. The acid degradate was isolated and its structure was elucidated by IR, ^{1}H-NMR and MS spectroscopy. This was followed by the development of five stability indicating spectroscopic and chromatographic methods for the determination of FLU in pure form. In addition, spectrofluorimetric method was adopted, based on the native fluorescence of FLU. The proposed methods were applied successfully with excellent accuracy and precision to the determination of FLU in pharmaceutical dosage form with satisfactory statistical validation measures.
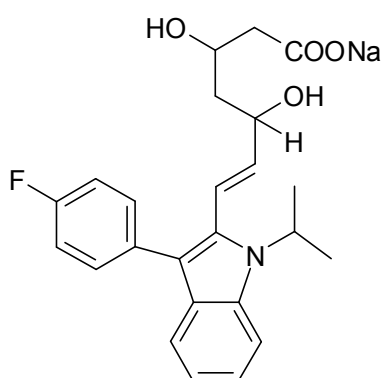


Mol. Formula $C_{24}H_{25}FNNaO_4$
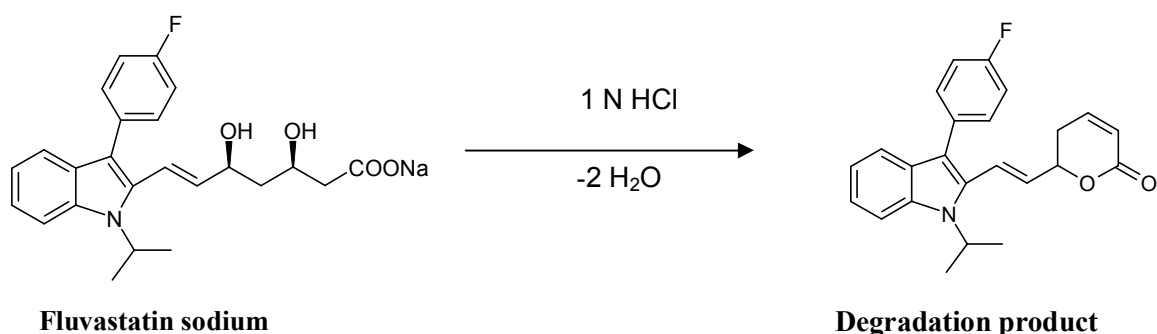
Mol. Wt. 433.45

**Fig. 1. Fluvastatin Sodium**

**Fig. 2. Suggested pathway for the degradation of Fluvastatin Sodium in 1 N HCl.**

## 2. Experimental

### 2.1. Instruments

1- A double-beam Shimadzu (Japan) 1601 PC UV-visible spectrophotometer connected to a computer fitted with UVPC personal spectroscopy software version 3.9 (Shimadzu), was used for the derivative and ratio spectra derivative spectrophotometry for measurements and treatment of data. The spectral band width was 2 nm with fast wavelength-scanning speed.

2- A Shimadzu RF – 1501 Spectrofluorophotometer. The excitation and emission scanning range was 220 – 900 nm with ordinate range limit 0 - 1000 and super scan speed.

3- A Schimadzu dual wavelength flying spot scanning densitometer Model CS – 9301 PC (Japan) was used for TLC-densitometric method. The experimental conditions of measurements were:
- Wavelength: 304 nm for FLU.
- Photomode: reflection.
- Scan mode: zigzag.
- Result output: chromatogram and area under the peak.

The TLC plates were 10 X 10 cm, precoated with silica gel, $F_{254}$, 0.2 mm thickness, purchased from Merck (Germany). The samples were applied to the plates using 25 μl Hamilton syringe.

4- UV lamp with short wavelength 254 nm (USA).

5- HPLC (Agilent 1100 series) instrument is equipped with an isocratic pump G1310A, a manual injector G1328B with a 20 μL loop and a UV – visible variable wavelength detector. The separation and quantitation were made on Agilent Zorbax $C_{18}$ column, 5 μm particle size (4.6 X 250 mm). Methanol: water (80 : 20, v/v) was used as mobile phase, filtered using 0.45 μm membrane filter and degassed by ultrasonic vibration. The flow rate was maintained at 1 mL.min$^{-1}$ and isocratic elution was applied throughout the analysis, with UV detection at 242 nm. The samples were filtered using 0.45 μm membrane filter and injected (20 μL) with a 25 μL Agilent analytical syringe. All determinations were performed at ambient temperature.

6- Ultrasonic processor: Soniclean, Thebarton SA, Australia.

7- Double distillator: Aquatron.

8- pH meter : Jenway, 3505, Essex, U.K.

9- A Chirana mechanical shaker, TE 111, K 0410 83a – 6206 – S, 140 rpm (Czechoslovakia).

10- Burker FT-IR spectrophotometer Vector 22, Schimadzu 435, Perkin-Elmer 457 and Jasco FT-IR plus 460 Japan, using potassium bromide discs.

11- Varian Gemini 200 MHZ, Joel Fx 90Q, 90 MHz FT spectrophotometer and Joel Ex 270 MHz spectrophotometer. The chemical shifts were expressed in δ ppm units, using trimethylsilane as the internal standard.

12- Hewlett Packard 5988 mass spectrometer at 70 eV.

### 2.2. Materials

#### 2.2.1. Pure standard

*Fluvastatin sodium* was kindly supplied by Novartis Pharma S.A.E., Cairo, under licence from Novartis Pharma AG., Basle, Switzerland. It was analyzed and found to be 99.11 ± 0.775 using RP-HPLC method[1].

#### 2.2.2. Pharmaceutical dosage form

**Lescol® XL tablets** produced by Novartis Pharma S.A.E., Cairo, under licence from Novartis Pharma AG., Basle, Switzerland. Batch No. 068/S0026. Each prolonged release tablet was labelled to contain 84.24

mg fluvastatin sodium equivalent to 80 mg fluvastatin free acid.

### 2.2. 3. Degraded sample

An accurately weighed 0.2 gm of Flu was dissolved in 10 mL water and refluxed with 50 mL 1 N HCl for about 90 min at 100°C. The colored precipitate formed was filtered, washed several times with water, dried in an oven at 50°C and left to cool in a dessicator.

The degradation product was tested by dissolving a part of the precipitate in methanol, spotting on a TLC plate next to a spot of Flu and allowing the plate to develop using chloroform : hexane : methanol : glacial acetic acid (5 : 5 : 1 : 1, v/v/v/v). By examining the TLC plate, two different spots were obtained, one for the intact drug ($R_f$ = 0.6) and the other for its acid degradation product ($R_f$ = 0.75). The degradation product of Flu was identified using IR, $^1$H-NMR and mass spectroscopy.

### 2.2.4. Chemicals, solvents and reagents

All chemicals used throughout this work were of analytical grade and the solvents were of spectroscopic grade.
1. Methanol : analar, Fisons, England, for spectroscopic and TLC methods and HPLC grade, sds, France, for HPLC method.
2. Chloroform, glacial acetic acid, ethanol 95 % and hexane were of analytical grade.
3. Hydrochloric acid : 37 %, Riedel-de Haën, Germany.
4. Water : double distilled water.
5. Sodium benzoate : BDH, England.
6. 1N HCl solution.

### 2.3. Standard solutions

**1. For third derivative ($^3$D), first and second derivative ratio ($^1$DD, $^2$DD) spectrophotometric methods :**
- Working standard solution of Flu (200 µg.mL$^{-1}$) in methanol was prepared from a stock solution of Flu (1 mg.mL$^{-1}$) in methanol.
- Acid degradate solution (0.4 mg.mL$^{-1}$) in methanol.
  **2. For spectrofluorimetric method :**
- Working standard solution of Flu (20 µg.mL$^{-1}$) in ethanol was prepared from a stock solution of Flu (0.2 mg.mL$^{-1}$) in ethanol.
  **3. For TLC-densitometric method :**
- Flu standard solution (2 mg.mL$^{-1}$) in methanol.
- Acid degradate solution (2 mg.mL$^{-1}$) in methanol.
  **4. For High performance liquid chromatographic (HPLC) method :**
- Working standard solution of Flu (100 µg.mL$^{-1}$) in mobile phase was prepared from a stock solution of Flu (1 mg.mL$^{-1}$) in mobile phase.
- Sodium benzoate solution as internal standard (IS) (0.4 mg.mL$^{-1}$) in mobile phase.

- Acid degradate solution (0.1 mg.mL$^{-1}$) in mobile phase.

### 2.4. Laboratory prepared mixtures

Different mixtures containing 10 – 90 % of the acid degradate were prepared from Flu working standard solutions. For HPLC method, 1 mL of sodium benzoate solution (0.4 mg.mL$^{-1}$) was added as an IS to each flask.

## 3. Procedures
### 3.1. Spectroscopic methods
### 3.1.1. Third derivative ($^3$D) method
### 3.1.1.1. Spectral characteristics of FLU and its degradate

Two aliquots (1 mL and 0.5 mL, respectively) of both FLU working standard and its degradate solutions (200 µg.mL$^{-1}$ and 0.4 mg.mL$^{-1}$, respectively) were, separately, transferred into two 10 mL volumetric flasks. The volume was completed with methanol to obtain 20 µg mL$^{-1}$ final concentration for each. The zero-order and the first-derivative spectra of the prepared solutions were recorded.

### 3.1.1.2. Linearity

Portions equivalent to (0.2–1 mL) of FLU working standard solution (200 µg mL$^{-1}$) were separately transferred to a series of 10 mL volumetric flasks. Each flask was completed to the volume with methanol to reach the concentration range of 4–20 µg mL$^{-1}$. The amplitudes of the third derivative peaks were measured at 318.6 nm with $\Delta\lambda$ = 16 nm and a scaling factor = 500. Calibration graphs were constructed by plotting the amplitudes of the maxima at 318.6 nm *versus* concentrations. The regression equation was then computed for the studied drug at the specified wavelength and used for determination of unknown samples containing FLU.

### 3.1.2. First and second derivative ratio spectrophotometric ($^1$DD, $^2$DD) methods
### 3.1.2.1. Linearity

Standard serial concentrations in the range of 4–20 µg mL$^{-1}$ aqueous solutions of FLU were prepared as under Section 3.1.1.2. The normalized spectrum of the acid degradate solution (1 µg mL$^{-1}$) was used as a divisor. The spectra of the prepared standard solutions were scanned (200–400 nm) and stored into the PC. The stored spectra of FLU were divided (amplitude at each wavelength) by the normalized spectrum of the acid degradate. The first and second derivatives of the ratio spectra were obtained and smoothed at $\Delta\lambda$ = 4 nm and scaling factor 10 and at $\Delta\lambda$ = 8 nm and scaling factor 50, respectively. The amplitudes of the minima of the first-derivative peaks of FLU at 259.4 nm ($^1$DD$_{259.4}$) and that of the maxima at 240.4 nm

($^1DD_{240.4}$) and 294.8 nm ($^1DD_{294.8}$) ($^1DD$ method) and that of the minima at 250.4 nm ($^2DD_{250.4}$) and that of the maxima at 264.2 nm ($^2DD_{264.2}$) ($^2DD$ method) were measured. Calibration graphs were constructed relating the peak amplitudes of ($^1DD$) and ($^2DD$) to the corresponding concentrations. The regression equations were then computed for the studied drug at the five specified wavelengths and used for the determination of unknown samples containing FLU.

### 3.1.3. Spectrofluorimetric method
#### 3.1.3.1. Determination of the wavelengths of excitation and emission
An aliquot (4 mL) of FLU working standard solution (20 µg.mL$^{-1}$) was transferred into a 10 mL volumetric flask and the volume was completed with ethanol. The excitation and emission spectra of the solution were recorded against ethanol as blank.

#### 3.1.3.2. Linearity
Accurately measured aliquots (0.5 – 5 mL) of FLU working standard solution (20 µg.mL$^{-1}$) were transferred into a series of 10 mL volumetric flasks to obtain the concentration range (10 – 100 µg) and the volume was completed with ethanol. The relative fluorescence intensity of each solution was recorded against the solvent blank, after excitation at 258 nm. Calibration curve was constructed relating the relative fluorescence intensities and the corresponding concentrations of FLU and the regression equation was computed.

### 3.2. Chromatographic methods
### 3.2.1. TLC-densitometric method
#### 3.2.1.1. Linearity
Accurate aliquots (0.5 – 5 mL) of FLU standard solution (2 mg.mL$^{-1}$) equivalent to (1 – 10 mg) were transferred into a series of 10 mL volumetric flasks and the volume was completed with methanol. An aliquot of 10 µl of each solution was applied to a TLC plate, as compact spots, 1.5 cm apart from each other and 1 cm from the bottom edge of the plate, using 25 µL Hamilton syringe. Ascending chromatography, through a distance of 8 cm, was performed in a chromatographic tank previously saturated with chloroform : hexane : methanol : glacial acetic acid (5:5:1:1, v/v/v/v) for 1 hour. The developed plates were dried at room temperature, then, the spots were detected under UV lamp (254 nm) and scanned with the spectrodensitometer at 304 nm for FLU. A calibration curve was constructed by plotting the recorded areas under the peaks versus the corresponding concentrations of FLU. The regression equation was then computed for the studied drug and used for determination of unknown samples containing it.

### 3.2.2. High performance liquid chromatographic method (HPLC)
#### 3.2.2.1. Linearity
Accurate aliquots (0.5 – 5 mL) of FLU working standard solution (100 µg.mL$^{-1}$) equivalent to (50 – 500 µg) were transferred separately into a series of 10 mL volumetric flasks and mixed with 1 mL of sodium benzoate solution (0.4 mg.mL$^{-1}$) as an IS. The volume was completed with the mobile phase to get the concentrations of (5 - 50 µg.mL$^{-1}$). The samples were injected onto the column (20 µL) and chromatographed using the specified chromatographic conditions mentioned under [2.1.4-]. The resulting chromatograms, retention times and the areas under the peaks were recorded and the peak area ratios of FLU to that of the IS were calculated.

The calibration curve representing the relationship between the peak area ratios and the corresponding concentrations of FLU were plotted and the regression equation was computed.

### 3.3. Analysis of laboratory prepared mixtures containing different ratios of FLU and its acid degradate using the suggested methods
Aliquots of intact drug and the degraded drug were mixed to prepare different mixtures containing 10–90% (w/w) of the acid degradate and the procedure was followed as mentioned under each method. The concentrations of FLU were calculated from the corresponding regression equations.

### 3.4. Application to pharmaceutical dosage form
An accurately weighed amount of the powdered tablets equivalent to either 25 mg FLU (for $^3D$, $^1DD$, $^2DD$, TLC) or 10 mg (for spectrofluorimetry) or 2.5 mg (for HPLC) were transferred into either 25 mL (for $^3D$, $^1DD$, $^2DD$, TLC, HPLC) or 50 mL volumetric flask (for spectrofluorimetry). The appropriate solvent was added (15 mL methanol for $^3D$, $^1DD$, $^2DD$, TLC, HPLC and 30 mL ethanol for spectrofluorimetry), shaken mechanically for 30 min, completed to volume with the appropriate solvent, mixed well and filtered on dry funnel and dry filter paper, discarding the first few milliliters. The solutions were diluted to the same concentrations of the appropriate working solutions and proceded according to the procedure of each method previously mentioned.

## 4. Results and discussion
### 4.1. Degradation of Flu
No data about the degradation pathway as well as the chemical structure of potential degradation product of FLU have been reported. Therefore, forced stability

study under stress conditions were carried out and revealed the instability of the drug in acid medium. When FLU was dissolved in water and refluxed with 50 mL 1 N HCl at 100 °C for 90 min, a colored precipitate was formed, indicating degradation of the drug. Different concentrations of HCl were tried and it was found that 0.1 N HCl was capable to produce degradation but 1 N HCl solution was used for the preparation of the degradation product to ensure complete degradation in a short time. This was demonstrated using TLC plate. In contrast to acid instability, alkaline conditions had no effect on decomposition of FLU.

The melting point of the degradation product was tested and found to be 160 °C.

The suggested pathway for the degradation of FLU includes dehydration and lactonization, as shown in Fig. 2.

The assignment of the acid degradate was based on the comparison of the IR and ^{1}H-NMR spectral data with those of the intact drug. Moreover, the molecular weight of the acid degradation product was confirmed by mass spectroscopy.

The IR spectrum (KBr) of intact FLU is characterized by the absorption frequency of two OH stretching band at 3420.3 cm$^{-1}$ and that of C=O at 1653.7 cm$^{-1}$. By contrast, the IR spectrum (KBr) of the acid degradation product revealed the C=O at 1699.8 cm$^{-1}$, indicating a change of its nature, while it lacked the characteristic OH stretching band at 3420 cm$^{-1}$ of the intact drug, Fig. 3.

The ^{1}H-NMR spectrum of intact FLU in chloroform (CDCl$_3$) was characterized by the appearance of the signals of the protons of the two methyl groups at δ 1.346 ppm, two methylene group of the 6- heptenoate as a multiplet at δ 2.207 – 2.423 ppm, the three protons –C<u>H</u>-N and 2 –C<u>H</u>-OH as multiplet at δ 4.567 ppm, 2 OH at δ 4.274 ppm which disappears on deuteration, the two protons of the double bond of the side chain as a doublet at δ 5.496 – 6.536 ppm and the eight aromatic protons as a multiplet at 6.86 – 7.391 ppm.

The ^{1}H-NMR spectrum of the acid degradation product in the same solvent showed the disappearance of the 2 OH signals with the appearance of two new multiplets at δ 6.351-6.796 ppm corresponding to C<u>H</u>=C<u>H</u> of the lactone ring, Fig. 4. On the other hand, MS of the degradation product showed the molecular ion peak (M$^+$) at 375, Fig. 5.

From the above spectral data, degradation behaviour is attributed to the extreme lability of the β and γ hydroxy groups on the heptenoic acid side chain and the presence of the double bond, while the basic ring nucleus remained the same. Therefore, it is assumed that FLU molecule is readily dehydrated by 1 N HCl and rearranged after the loss of two molecules of water to give the degradation product, illustrated in

Fig. 2, whose molecular weight was confirmed by (M$^+$) in mass spectrum.

On account of acid instability, oral dosage forms comprising FLU are stabilized against pH-related degradation by an alkaline stabilizing medium, such as carbonate salts, which are capable of imparting a pH of ≥ 8 to an aqueous solution or dispersion of the compound[20]. However, the local alkaline environment created at the site of dissolution of the dosage form has a negative effect on gastric mucosa[21]. Therefore, stabilized pharmaceutical compositions comprising FLU, were developed by utilizing either cyclodextrin[21,22] as an inclusion complexing agent or dimethicone as a protective barrier[23], in order to protect the drug from destabilization in acidic environment. The focus of the present work was to develop accurate, specific, reproducible and sensitive stability indicating methods for the determination of FLU in pure form or in pharmaceutical formulations in the presence of its acid degradate product.

### 4.2. Spectroscopic methods
### 4.2.1. Third derivative ($^3$D) method

Direct UV absorption measurements were found to be inapplicable to the analysis of FLU in presence of its acid degradate because of the spectral interference, Fig. 6.

A simple, rapid and selective derivative spectrophotometric technique was proposed and applied for the determination of FLU in the presence of its acid degradate, either in pure form or in pharmaceutical formulation. This was done by applying the third derivative ($^3$D) UV spectrophotometry. The method can solve the problem of spectral bands overlapping between FLU and its acid degradate without sample pretreatment or separation steps of the analyzed drug and its acid degradate.

The more convenient order of derivative and working wavelength were selected by preliminary tests. The third derivative spectra proved to be optimal as it presented spectral features which can be used for the determination of FLU in presence of its acid degradate. Optimum results were obtained using Δ λ = 16 and scaling factor 500.

As shown in Fig. 7, the overlapping observed in the zero–order absorption spectra was eliminated. A sharp peak at 318.6 nm ($^3$D$_{318.6}$) for the intact molecule, which lies at the zero-crossing of the acid degradate, was obtained. Therefore, the peak amplitude at 318.6 nm (H$_{318.6}$) was used for the quantitation of FLU in presence of up to 70 % of the acid degradate without any prior separation procedures. A linear correlation was obtained in the range of 4 – 20 μg.mL$^{-1}$ for FLU.

The regression equation was computed and found to be:

$$H_{318.6} = 3.4303 \, C - 0.0027$$

r = 0.9994, at 318.6 nm.

Where $H_{318.6}$ is the amplitude of the maximum at 318.6 nm ($^3D_{318.6}$) x $10^3$,

C is the concentration of FLU ($\mu g.mL^{-1}$) and

r is the correlation coefficient.

The precision of the proposed method was confirmed by the analysis of different concentrations of authentic samples in triplicates. The mean percentage recoveries were found to be 100.08 ± 0.779.

### 4.2.2. First and second derivative ratio spectrophotometric ($^1DD$, $^2DD$) methods

The derivative ratio spectroscopy is a useful tool in quantification of drugs. It permits the determination of FLU at the wavelengths corresponding to a maximum or minimum, thus, leading to better sensitivity than the zero-crossing method in derivative spectrophotometry and without previous separation[24-26]. It could be applied as a stability-indicating method for the determination of FLU in presence of up to 80 % of its acid degradate, which is a higher degradation percentage than the third derivative method does.

The main instrumental parameters affecting the shape of derivative ratio spectra are $\Delta\lambda$ and scaling factor[27]. These parameters need to be optimsed to give good selectivity, higher sensitivity and an adequate signal-to-noise ratio. An accurate choice of standard divisor and working wavelength are of capital importance[26,28,29] hence, the method was tested with various divisor concentrations. Normalized spectrum of the acid degradate was selected as divisor for the determination of FLU. It was obtained by dividing the spectra of several standards of variable concentration by their corresponding concentrations and subsequently averaging them, in order to obtain a spectrum of unit concentration. The use of normalized spectrum assured the best compromise in terms of sensitivity, repeatability and signal to noise ratio. In addition, it diminishes the quantitation errors because of elimination of most random noise through averaging[30].

The stored spectra of different concentrations of FLU were divided by the normalized spectrum of its acid degradate and the first and second derivatives of the ratio spectra were obtained and smoothed at $\Delta\lambda = 4$ nm and scaling factor 10 and at $\Delta\lambda = 8$ nm and scaling factor 50, respectively, Fig. 8.

A linear response was obtained between the peak amplitudes at 240.4 nm ($^1DD_{240.4}$), 259.4 nm ($^1DD_{259.4}$) and 294.8 nm ($^1DD_{294.8}$) ($^1DD$ method) and at 250.4 nm ($^2DD_{250.4}$) and 264.2 nm ($^2DD_{264.2}$) ($^2DD$ method) and the corresponding drug concentrations in the range of 4 - 20 $\mu g.mL^{-1}$. The regression equations were computed and found to be :

### a) First derivative ratio method:

$^1DD_{240.4} = \phantom{-}0.3231C - 0.0275 \qquad$ r = 0.9999, $\qquad$ at 240.4 nm.

$^1DD_{259.4} = -0.6035C - 0.0401 \qquad$ r = 0.9998, $\qquad$ at 259.4 nm.

$^1DD_{294.8} = \phantom{-}0.1974C + 0.0130 \qquad$ r = 0.9998, $\qquad$ at 294.8 nm

### b) Second derivative ratio method :

$^2DD_{250.4} = -0.3456C - 0.0083 \qquad$ r = 0.9992, $\qquad$ at 250.4 nm.

$^2DD_{264.2} = \phantom{-}0.3146C - 0.0239 \qquad$ r = 0.9994, $\qquad$ at 264.2 nm.

Where $^1DD$ is the peak amplitude of the first derivative of the ratio spectra,

$^2DD$ is the peak amplitude of the second derivative of the ratio spectra,

C is the concentration of FLU ($\mu g.mL^{-1}$) and r is the correlation coefficient.

The precision of the proposed methods was checked by the analysis of different concentrations of authentic samples in triplicates. The mean percentage recoveries were found to be $99.94 \pm 0.836$, $99.77 \pm 0.672$ and $99.48 \pm 0.819$ at 240.4 nm ($^1DD_{240.4}$), 259.4 nm ($^1DD_{259.4}$) and 294.8 nm ($^1DD_{294.8}$), respectively, in case of $^1DD$ method and $100.02 \pm 0.587$ and $99.47 \pm 0.688$ at 250.4 nm ($^2DD_{250.4}$) and 264.2 nm ($^2DD_{264.2}$), respectively, in case of $^2DD$ method.

### 4.2.3. Spectrofluorimetric method

Fluorescence spectroscopy is widely used in chemical analysis owing to its high selectivity and relatively low cost[31-32]. In addition, one of the most attractive features of molecular fluorescence is its inherent sensitivity which is often one to three orders of magnitude better than absorption spectroscopy[33].

FLU showed native fluorescence in ethanol, methanol, 0.01 N sodium hydroxide and 0.01 N sulfuric acid. However, ethanol was chosen as solvent because the drug solution in ethanol exhibited the strongest fluorescence intensity at 776 nm when excited at 258 nm, Fig. 9.

This fluorescence property of FLU was used for the determination of pure samples of the drug in concentrations ranging from $1 - 10$ µg.mL$^{-1}$. A linear correlation was obtained between the relative fluorescence intensity and concentration of the drug over this range and a linear regression equation was computed and found to be:

$F_{776} = 89.0958\,C + 110.9595$
$r = 0.9996.$.

Where $F_{776}$ is the relative fluorescence intensity of FLU at 776 nm with excitation at 258 nm,
C is the concentration of FLU (µg.mL$^{-1}$) and
r is the correlation coefficient.

By this equation, pure samples of FLU were quantitatively determined with mean percentage recovery $100.24 \pm 0.957$.

### 4.3. Chromatographic methods
### 4.3.1. TLC-densitometric method

Instrumental planar chromatography, with precise sample application and computer controlled evaluation and quantification of the developed chromatograms, has been considered as reliable tool for purity control and quantitative drug testing. Quantitative methods depending on measuring the optical density of the separated spots on a TLC plate have been described[29, 34-36].

The objective of this work was to establish a TLC–densitometric method as a stability indicating method for the determination of FLU in presence of its acid degradate with satisfactory precision enough for good analytical practice. The proposed method was based on the significant TLC separation of FLU ($R_f = 0.6$) and the acid degradate ($R_f = 0.75$).

Different developing systems were tried for complete separation. The best resolution was achieved by using chloroform : hexane : methanol : glacial acetic acid (5 : 5 : 1 : 1, v/v/v/v), as developing system. The spots developed were dense and compact and the resolved spot of FLU was scanned at 304 nm, Fig. 10.

The linearity was confirmed by plotting the measured areas under the peaks versus the corresponding concentrations at 304 nm in the range of $1 - 10$ µg/spot of FLU. The regression equation was computed and found to be :

$AUP = 0.3663\,C + 0.6823$
$r = 0.9991.$
Where AUP is the area under the peak x $10^{-3}$,
C is the concentration of FLU (µg/spot) and
r is the correlation coefficient. By this equation, it was possible to determine FLU pure samples with mean percentage recovery $100.07 \pm 0.935$.

### 4.3.2. High performance liquid chromatographic method (HPLC)

HPLC is the technique most widely used for the quantitative determination of drugs present either alone or in mixture in one single procedure[37-40]. The objective of this research was to develop and validate a rapid and sensitive and stability indicating isocratic HPLC method for the quantitative determination of FLU in presence of its acid degradate, either in bulk powder or in pharmaceutical dosage form.

Several trials have been carried out to obtain a good separation between the intact drug and its acid degradate. These trials involved the use of different mobile phases with different ratios and flow rates. A complete baseline separation of FLU and its acid degradate and peak symmetry were obtained using $C_{18}$ column with a mobile phase consisting of methanol : water in the ratio of (80 : 20, v/v) at ambient temperature and flow rate of 1 mL.min$^{-1}$. Quantitation was achieved, with UV detection at 242 nm, based on peak area and sodium benzoate was used as an IS.

The specificity of the HPLC method is illustrated in Fig. 11, where complete separation of FLU and its acid degradate is noticed. The average retention time $\pm$ S.D. and the order of elution were found to be $1.896 \pm 0.004$, $3.335 \pm 0.014$ and $7.483 \pm 0.092$, in case of sodium benzoate (IS), FLU and its acid degradate, respectively, with an overall analysis time of about 10 min.

The calibration curve representing the relation between the concentrations of Flu versus the peak area ratios was constructed, a linear relationship in the range of $5 - 50$ µg.mL$^{-1}$ was obtained. The regression equation was computed and found to be :

$Y = 0.1206\,C - 0.0431$
$r = 0.9990.$

Where C is the concentration ($\mu g.mL^{-1}$),
Y is the peak area ratio (= peak area of the sample / peak area of IS) and
r is the correlation coefficient.

An accuracy study of the method was performed, with mean percentage recovery $100.17 \pm 0.916$ of FLU, demonstrating that the method was accurate.

System suitability parameters were tested according to USP 30 [1] by calculating tailing factor, column capacity, column efficiency, the height of the theoretical plate (H), resolution and relative retention from the chromatogram of FLU and its acid degradate, Fig. 6. The system was found to be suitable, as shown in Table 1.

### 4.4 Stability indication

To assess the stability indicating efficiency of the proposed methods, the acid degradate of FLU was mixed with its intact sample in different ratios and analyzed by the proposed methods. Table 2 illustrates good selectivity in the determination of FLU in the presence of up to 70% (w/w) of its acid degradate in the $^3$D method, up to 80% (w/w) by the $^1$DD, $^2$DD, TLC-densitometric and HPLC methods.

### 4.5. Analysis of pharmaceutical dosage form

The suggested methods were successfully applied for the determination of FLU in its pharmaceutical formulation, showing good percentage recoveries. The validity of the suggested methods was further assessed by applying the standard addition technique, as presented in Table 3.

### 4.6. Method validation

Method validation was performed according to USP guidelines[1] for all the proposed methods. The precision of the suggested methods was also expressed in terms of relative standard deviation of the interday and intraday analysis results. Table 4 shows results of accuracy, precision, LOD and LOQ of the methods.

### 4.7. Statistical analysis

Results of the suggested methods for determination of FLU were statistically compared with those obtained by applying the official HPLC method[1]. The calculated *t*- and *F*- values were found to be less than the corresponding theoretical ones, confirming good accuracy and excellent precision, Table 5. Furthermore, One-way ANOVA at $p < 0.05$[41-43] was applied for the comparison of these methods where there is no significant difference between the proposed methods and the official method as the p-value is greater than 0.05, Table 6 and Fig. 12.

### 5. Conclusion

The suggested methods provide simple, accurate and reproducible quantitative analysis of FLU in pure sample, pharmaceutical formulation and in presence of its acid degradate.

Reviewing literature, no methods were concerned with the determination of FLU in presence of its acid degradate which was not identified and no synthetic mixtures were prepared to check the specificity of the methods.

The spectrofluorimetric method was more sensitive than the other methods. While the $^3$D, $^1$DD and $^2$DD methods are well-established techniques that are able to enhance the resolution of overlapping bands. These methods are simple, more convenient, less time consuming and economic stability indicating methods compared to other published LC methods.

The advantages of TLC-densitometric method is that several samples can be run simultaneously using minimal volume of solvents, compared with HPLC method, thus lowering analysis time and cost per analysis and provides high sensitivity and selectivity. The HPLC method gives a good resolution between FLU and its acid degradate with a short analysis time.

High values of correlation coefficients and small values of intercepts validated the linearity of the calibration graphs and the obedience to Beer's law. The R.S.D. values, the slopes and the intercepts of the calibration graphs indicated the high reproducibility of the proposed methods.

From the results obtained, we concluded that the suggested methods showed high sensitivity, accuracy, reproducibility and specificity and can be used as stability indicating methods. Moreover, these methods are simple and inexpensive, permitting their application in quality control laboratories.

**Table 1 : Chromatographic parameters required for system suitability test in the determination of fluvastatin sodium in presence of its acid degradation product by RP-HPLC method.**

| Parameter | Obtained value | Reference value[28,332-334] |
|---|---|---|
| | **Fluvastatin sodium** | |
| Tailing factor (T) | 1.5 | $\leq 2$ |
| Column capacity (K) | 2.308 | 1 - 10 |
| Column efficiency (N) | 5421.941 | The higher the value, the more efficient the column is. |
| Height equivalent to theoretical plate (H) | 0.0046 cm/plate | The smaller the value, the higher the column efficiency |
| Resolution (R) | 12.18 $(a_1)$[*] <br> 10.85 $(a_2)$[*] | > 2 |
| Relative retention (α) | 2.80 $(b_1)$[**] <br> 2.54 $(b_2)$[**] | $\geq 1$ |

[*] $a_1$ and $a_2$ calculated for fluvastatin sodium – acid degradation product and fluvastatin sodium – IS, respectively.

[**] $b_1$ and $b_2$ calculated for fluvastatin sodium – acid degradation product and fluvastatin sodium – IS, respectively.

**Table 2: Determination of Fluvastatin Sodium in presence of its acid degradate in laboratory prepared mixtures by the proposed methods.**

| Degradate % | Recovery %[a] of FLU | | | | | | TLC - densitometric method | HPLC method |
|---|---|---|---|---|---|---|---|---|
| | [3]D method | [1]DD spectrophotometric method | | | [2]DD spectrophotometric method | | | |
| | 318.6 nm | 240.4 nm | 259.4 nm | 294.8 nm | 250.4 nm | 264.2 nm | | |
| 10 | 101.23 | 100.89 | 100.33 | 100.42 | 100.78 | 100.73 | 99.00 | 99.58 |
| 30 | 99.54 | 99.94 | 100.77 | 100.16 | 99.34 | 100.46 | 101.47 | 100.03 |
| 50 | 99.12 | 99.89 | 98.64 | 99.54 | 100.08 | 99.30 | 100.34 | 100.60 |
| 70 | 99.62 | 100.15 | 99.38 | 100.90 | 98.95 | 101.50 | 99.27 | 101.50 |
| 80 | 110.12[b] | 98.93 | 100.83 | 98.90 | 101.25 | 101.23 | 99.90 | 101.17 |
| 90 | 138.42[b] | 105.57[b] | 104.30[b] | 106.71[b] | 106.76[b] | 103.18[b] | 115.60[b] | 129.99[b] |
| Mean ± S.D. | 99.88 ± 0.928 | 99.96 ± 0.701 | 99.99 ± 0.952 | 99.98 ± 0.780 | 100.08 ± 0.960 | 100.64 ± 0.855 | 100.00 ± 0.977 | 100.58 ± 0.790 |

[a] Average of three determinations
[b] Rejected values.

**Table 3 Quantitative determination of Fluvastatin Sodium in Lescol® XL tablets by the proposed methods and application of standard addition technique.**

| The proposed method | Lescol® XL tablets (Batch No. 068/S0026) | Standard addition technique | | |
|---|---|---|---|---|
| | Recovery %[a] ± S.D. | Pure added ($\mu g.mL^{-1}$) | Pure found [a] ($\mu g.mL^{-1}$) | Recovery % |
| [3]D method At 318.6 nm | 99.49 ± 0.962 | 4 | 3.994 | 99.85 |
| | | 5 | 5.014 | 100.28 |
| | | 6 | 5.947 | 99.12 |
| | | 8 | 7.958 | 99.48 |
| | | 10 | 9.824 | 98.24 |
| | | 12 | 11.865 | 98.88 |
| Mean ± S.D. | | | | 99.31 ± 0.725 |
| [1]DD spectrophotometric method At 240.4 nm | 100.72 ± 0.629 | 4 | 4.014 | 100.35 |
| | | 5 | 4.977 | 99.54 |
| | | 6 | 5.964 | 99.40 |
| | | 8 | 8.010 | 100.13 |
| | | 10 | 10.006 | 100.06 |
| | | 12 | 12.046 | 100.38 |
| Mean ± S.D. | | | | 99.98 ± 0.414 |
| At 259.4 nm | 100.42 ± 0.514 | 4 | 4.036 | 100.90 |
| | | 5 | 5.025 | 100.50 |
| | | 6 | 5.995 | 99.92 |
| | | 8 | 8.041 | 100.51 |
| | | 10 | 10.006 | 100.06 |
| | | 12 | 11.874 | 98.95 |
| Mean ± S.D. | | | | 100.14 ± 0.680 |
| At 294.8 nm | 99.62 ± 0.519 | 4 | 4.022 | 100.55 |
| | | 5 | 5.015 | 100.30 |
| | | 6 | 6.104 | 101.73 |
| | | 8 | 8.100 | 101.25 |
| | | 10 | 10.096 | 100.96 |
| | | 12 | 12.209 | 101.74 |
| Mean ± S.D. | | | | 101.09 ± 0.598 |
| [2]DD spectrophotometric method At 250.4 nm | 100.03 ± 0.730 | 4 | 4.022 | 100.55 |
| | | 5 | 4.962 | 99.24 |
| | | 6 | 6.024 | 100.40 |
| | | 8 | 7.925 | 99.06 |
| | | 10 | 10.113 | 101.13 |
| | | 12 | 12.011 | 100.09 |
| Mean ± S.D. | | | | 100.08 ± 0.796 |
| At 264.2 nm | 99.79 ± 0.699 | 4 | 3.970 | 99.25 |
| | | 5 | 4.962 | 99.24 |
| | | 6 | 5.972 | 99.53 |
| | | 8 | 7.978 | 99.73 |
| | | 10 | 9.869 | 98.69 |
| | | 12 | 11.812 | 98.43 |
| Mean ± S.D. | | | | 99.15 ± 0.496 |
| Spectrofluorimetric method | 100.33 ± 0.807 | 2.0 | 1.999 | 99.95 |
| | | 3.0 | 2.956 | 98.53 |
| | | 4.0 | 3.962 | 99.05 |
| | | 5.0 | 5.001 | 100.02 |
| Mean ± S.D. | | | | 99.39 ± 0.722 |
| TLC -densitometric method | 99.45 ± 0.854 | 2[b] | 1.971[b] | 98.55 |
| | | 3[b] | 2.997[b] | 99.90 |
| | | 4[b] | 4.007[b] | 100.18 |
| | | 5[b] | 5.00[b] | 100.02 |
| | | 6[b] | 6.022[b] | 100.37 |
| Mean ± S.D. | | | | 99.80 ± 0.723 |
| HPLC method | 99.84 ± 0.298 | 10 | 9.975 | 99.75 |
| | | 15 | 14.851 | 99.01 |
| | | 20 | 20.257 | 101.29 |
| | | 25 | 24.784 | 99.14 |
| | | 30 | 29.702 | 99.01 |
| Mean ± S.D. | | | | 99.64 ± 0.972 |

[a] Average of three determinations  [b] ($\mu g$/spot)

**Table 4  Assay parameters and method validation obtained by applying the proposed methods for the determination of  Fluvastatin Sodium.**

| Parameters | [3]D method | [1]DD spectrophotometric method | | | [2]DD spectrophotometric method | | Spectrofluori-metric method | TLC - densitometric method | HPLC method |
|---|---|---|---|---|---|---|---|---|---|
| | 318.6 nm | 240.4 nm | 259.4 nm | 294.8 nm | 250.4 nm | 264.2 nm | | | |
| *Linearity* | | | | | | | | | |
| **Range** | 4 – 20 | | 4 - 20 | | | 4 – 20 | | 1 - 10 | 1 – 10 | 5 – 50 |
| | $\mu g.mL^{-1}$ | $\mu g.mL^{-1}$ | $\mu g.mL^{-1}$ | $\mu g.mL^{-1}$ | $\mu g.mL^{-1}$ | $\mu g.mL^{-1}$ | $\mu g.mL^{-1}$ | $\mu g/spot$ | $\mu g.mL^{-1}$ |
| **Slope (b)** | 3.4303 | 0.3231 | -0.6035 | 0.1974 | -0.3456 | 0.3146 | 89.0958 | 0.3663 | 0.1206 |
| **S.D. of slope ($S_b$)** | 0.032 | 0.001 | 0.003 | 0.001 | 0.004 | 0.003 | 0.674 | 0.005 | 0.002 |
| **Intercept (a)** | -0.0027 | -0.0275 | -0.0401 | 0.0130 | -0.0083 | -0.0239 | 110.9595 | 0.6823 | -0.0431 |
| **S.D. of intercept ($S_a$)** | 0.414 | 0.017 | 0.0430 | 0.0140 | 0.051 | 0.040 | 4.205 | 0.032 | 0.048 |
| **Correlation coefficient (r)** | 0.9994 | 0.9999 | 0.9998 | 0.9998 | 0.9992 | 0.9994 | 0.9996 | 0.9991 | 0.9990 |
| *Accuracy* | | | | | | | | | |
| **Mean** | 100.08 | 99.94 | 99.77 | 99.48 | 100.02 | 99.47 | 100.24 | 100.07 | 100.17 |
| **± S.D.** | ± 0.779 | ± 0.836 | ± 0.672 | ± 0.819 | ± 0.587 | ± 0.688 | ± 0.957 | ± 0.935 | ± 0.916 |
| *Specificity* | 99.88 ± 0.928 | 99.96 ± 0.701 | 99.99 ± 0.952 | 99.98 ± 0.780 | 100.08 ± 0.960 | 100.64 ± 0.855 | —— | 100.00 ± 0.977 | 100.58 ± 0.790 |
| *Precision* | | | | | | | | | |
| **Interday**[a] | 0.700-0.770 | 0.337-0.568 | 0.849-0.530 | 0.127-0.137 | 0.888-0.441 | 0.145-0.538 | 0.653 – 0.484 | 0.754-0.897 | 0.404-0.994 |
| **Intraday**[a] | 0.384-0.717 | 0.465-0.444 | 0.785-0.652 | 0.801-0.828 | 0.977-0.844 | 0.952-0.928 | 0.907 – 0.428 | 0.587-0.435 | 0.463-0.406 |
| **LOD**[b] | 0.19 | 0.17 | 0.23 | 0.19 | 0.45 | 0.19 | 0.09 | 0.09 | 0.51 |
| **LOQ**[c] | 0.57 | 0.50 | 0.71 | 0.58 | 1.36 | 0.56 | 0.26 | 0.29 | 1.55 |

[a] The interday (n=3) and intraday (n=3) relative standard deviations are for samples of concentrations (9, 15 $\mu g.mL^{-1}$), (3.6, 8.8 $\mu g.mL^{-1}$),(3.5, 7 $\mu g/spot$) and (25, 35 $\mu g.mL^{-1}$) for derivative and derivative ratio spectrophotometric, spectrofluorimetric, TLC-densitometric and HPLC methods, respectively.
[b] Limit of detection, calculated from the results of analysis of pure powder using the following equation [295,296]:  3.3(S.D./S) where, S.D. is the standard deviation of the response, S is the slope.
[c] Limit of quantification, calculated from the results of analysis of pure powder using the following equation: 10 (S.D./S) where, S.D. is the standard deviation of the response, S is the slope.

**Table 5 :Statistical comparison between the results obtained by applying the proposed methods and the reference method.**

| Prepara-tion | Statistical Term | Spectrophotometric methods | | | | | | Fluorimetric Method | TLC-densitometric Method | RP-HPLC Method | Reference Method[▲] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $^3$D At 318.6 nm | $^1$DD At 240.4 nm | $^1$DD At 259.4 nm | $^1$DD At 294.8 nm | $^2$DD At 250.4 nm | $^2$DD At 264.2 nm | | | | |
| Pure Sample | Mean ± S.D. | 100.08±0.779 | 99.94±0.836 | 99.77 ±0.672 | 99.48±0.819 | 100.02±0.587 | 99.47±0.688 | 100.24±0.957 | 100.07±0.935 | 100.17±0.916 | 99.11±0.775 |
| | n | 7 | 7 | 7 | 7 | 7 | 7 | 8 | 7 | 8 | 4 |
| | Variance | 0.607 | 0.699 | 0.452 | 0.671 | 0.345 | 0.473 | 0.916 | 0.874 | 0.839 | 0.601 |
| | S.E. | 0.294 | 0.316 | 0.254 | 0.310 | 0.222 | 0.260 | 0.338 | 0.353 | 0.324 | 0.388 |
| | Student's t | 1.993(2.262)* | 1.659 (2.262)* | 1.423(2.262)* | 0.745(2.262)* | 2.036(2.262)* | 0.771(2.262)* | 2.196(2.228)* | 1.830(2.262)* | 2.097(2.228)* | |
| | F ratio | 1.010 (8.94)* | 1.163 (8.94)* | 1.330 (4.76)* | 1.116 (8.94) * | 1.742 (4.76)* | 1.271 (4.76)* | 1.524 (8.88)* | 1.454 (8.94)* | 1.396 (8.88)* | |
| Lescol XL® Tablet | Mean ± S.D. | 99.49±0.962 | 100.72±0.629 | 100.42±0.514 | 99.62±0.519 | 100.03±0.730 | 99.79±0.699 | 100.33±0.807 | 99.45±0.854 | 99.84±0.298 | 100.32±0.847 |
| | n | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 3 | 3 | 4 |
| | Variance | 0.925 | 0.396 | 0.264 | 0.269 | 0.533 | 0.489 | 0.651 | 0.729 | 0.089 | 0.717 |
| | S.E. | 0.555 | 0.363 | 0.297 | 0.300 | 0.421 | 0.404 | 0.404 | 0.493 | 0.172 | 0.424 |
| | Student's t | 1.188(2.571)* | 0.717(2.571)* | 0.193(2.571)* | 1.348(2.571)* | 0.485(2.571)* | 0.905(2.571)* | 0.017(2.447)* | 1.338(2.571)* | 1.049(2.571)* | |
| | F ratio | 1.290 (9.55)* | 1.811(19.16)* | 2.716(19.16)* | 2.665(19.16)* | 1.345(19.16)* | 1.466(19.16)* | 1.101(9.28)* | 1.017 (9.55)* | 8.056(19.16)* | |

\* The values in the parenthesis are the corresponding values of t and F at (p=0.05).
▲ Reference method for pure fluvastatin sodium is RP-HPLC method ( using [28].

**Table 6:  Comparison between the results obtained by applying the proposed methods and the reference method for the analysis of Fluvastatin Sodium.**

| Methods | Pure sample | | Lescol XL® tablet | |
|---|---|---|---|---|
| | Mean | S.D. | Mean | S.D. |
| [3]D method | 100.08 | 0.779 | 99.49 | 0.962 |
| [1]DD method at 240.4 nm | 99.94 | 0.836 | 100.72 | 0.629 |
| [1]DD method at 259.4 nm | 99.77 | 0.672 | 100.42 | 0.514 |
| [1]DD method at 294.8 nm | 99.48 | 0.819 | 99.62 | 0.519 |
| [2]DD method at 250.4nm | 100.02 | 0.587 | 100.03 | 0.730 |
| [2]DD method at 264.2 nm | 99.47 | 0.688 | 99.79 | 0.699 |
| Fluorimetric method | 100.24 | 0.957 | 100.33 | 0.807 |
| TLC-densitometric method | 100.07 | 0.935 | 99.45 | 0.854 |
| RP-HPLC method | 100.17 | 0.916 | 99.84 | 0.298 |
| Reference method | 99.11 | 0.775 | 100.32 | 0.847 |
| F-value | 1.21 | | 1.11 | |
| p-value | 0.308 | | 0.398 | |

* = There is a significant difference between methods by using one way ANOVA at $p < 0.05$.
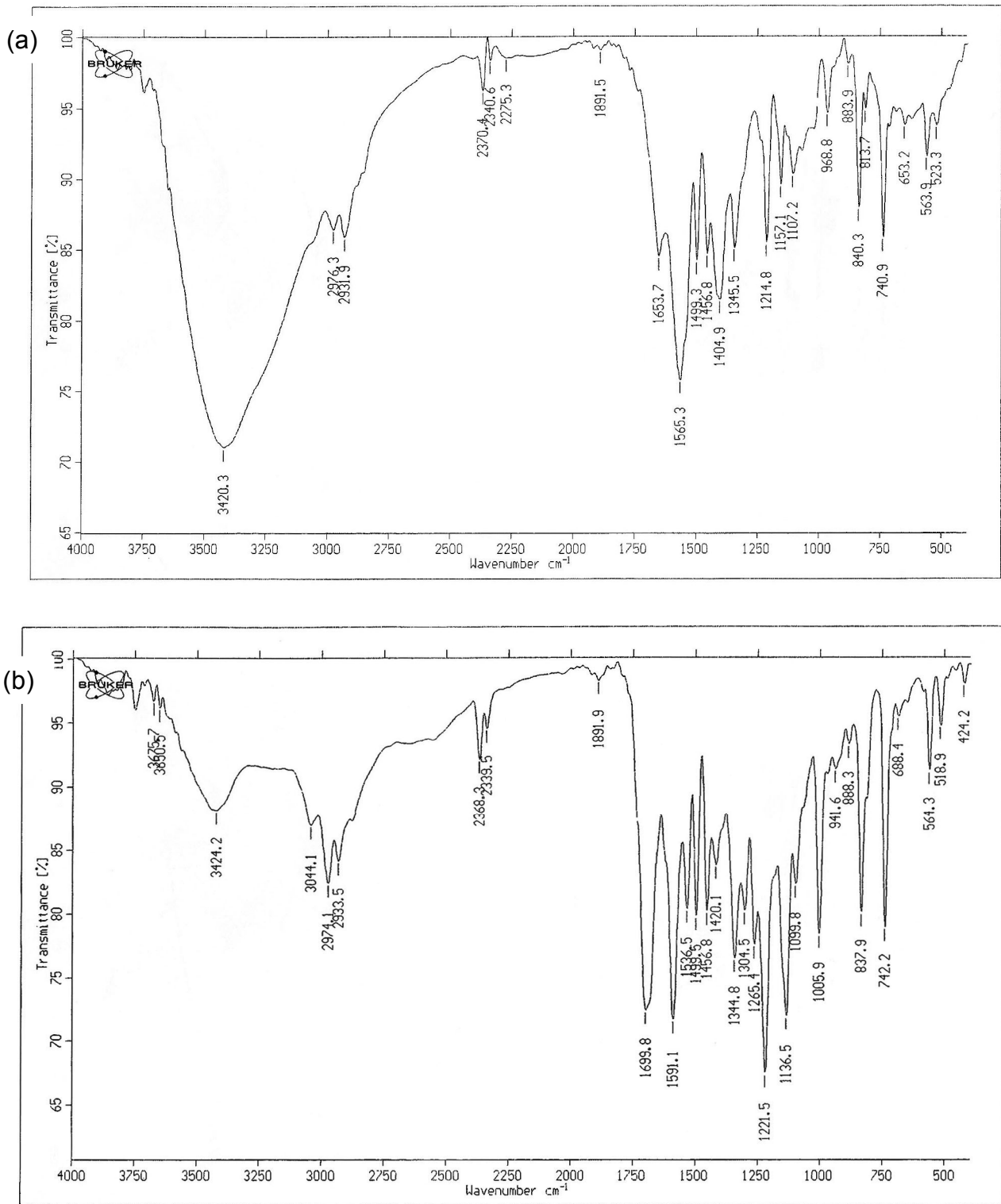
**Fig. 3. IR spectra of Fluvastatin Sodium (a) and its acid degradate (b).**
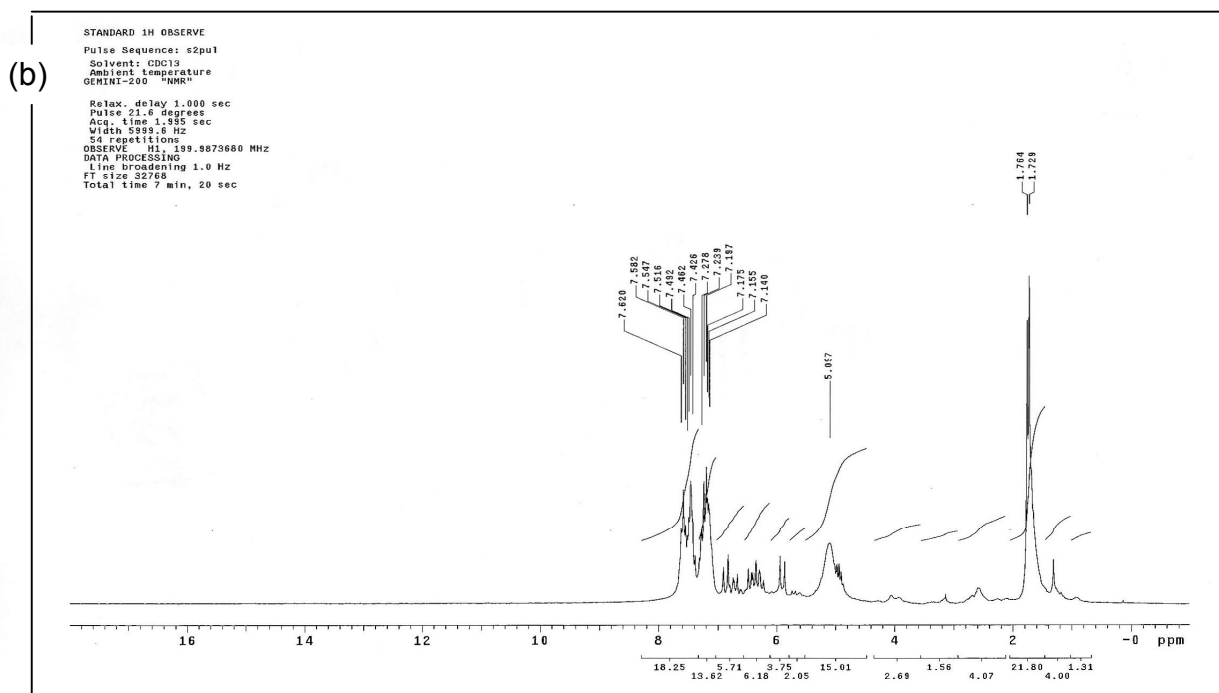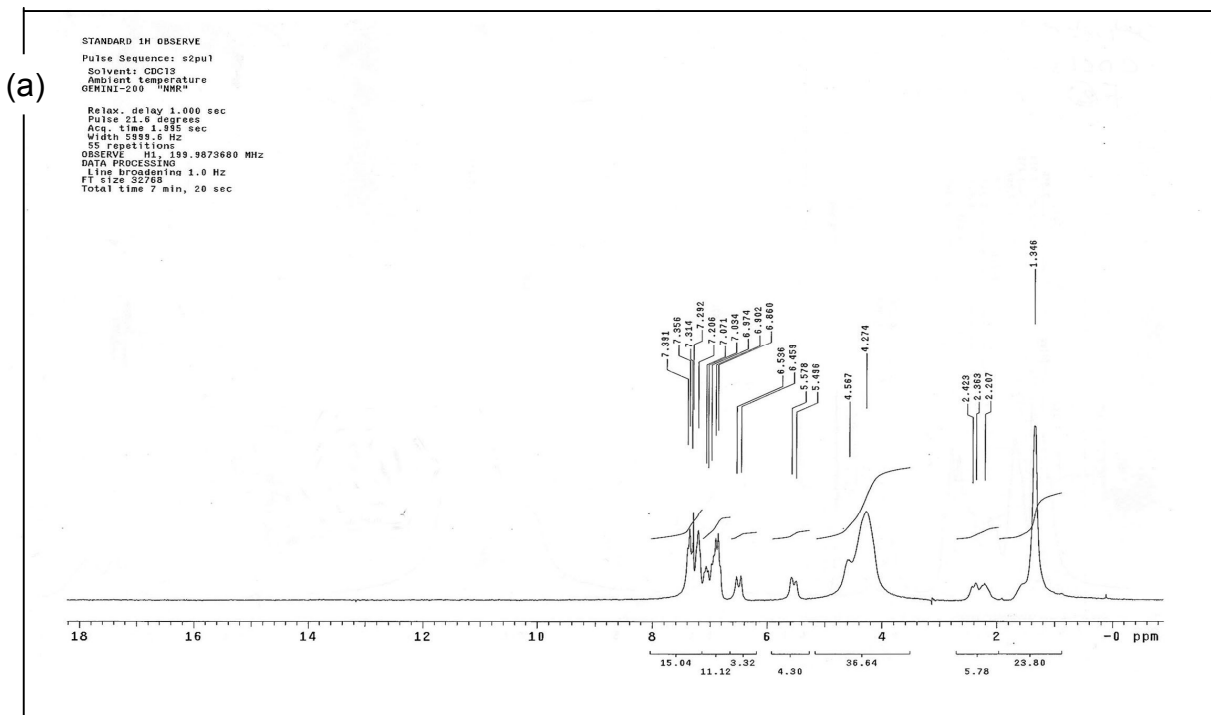
**Fig. 4. ¹H-NMR spectra of Fluvastatin Sodium (a) and its acid degradate (b) in chloroform (CDCl₃).**
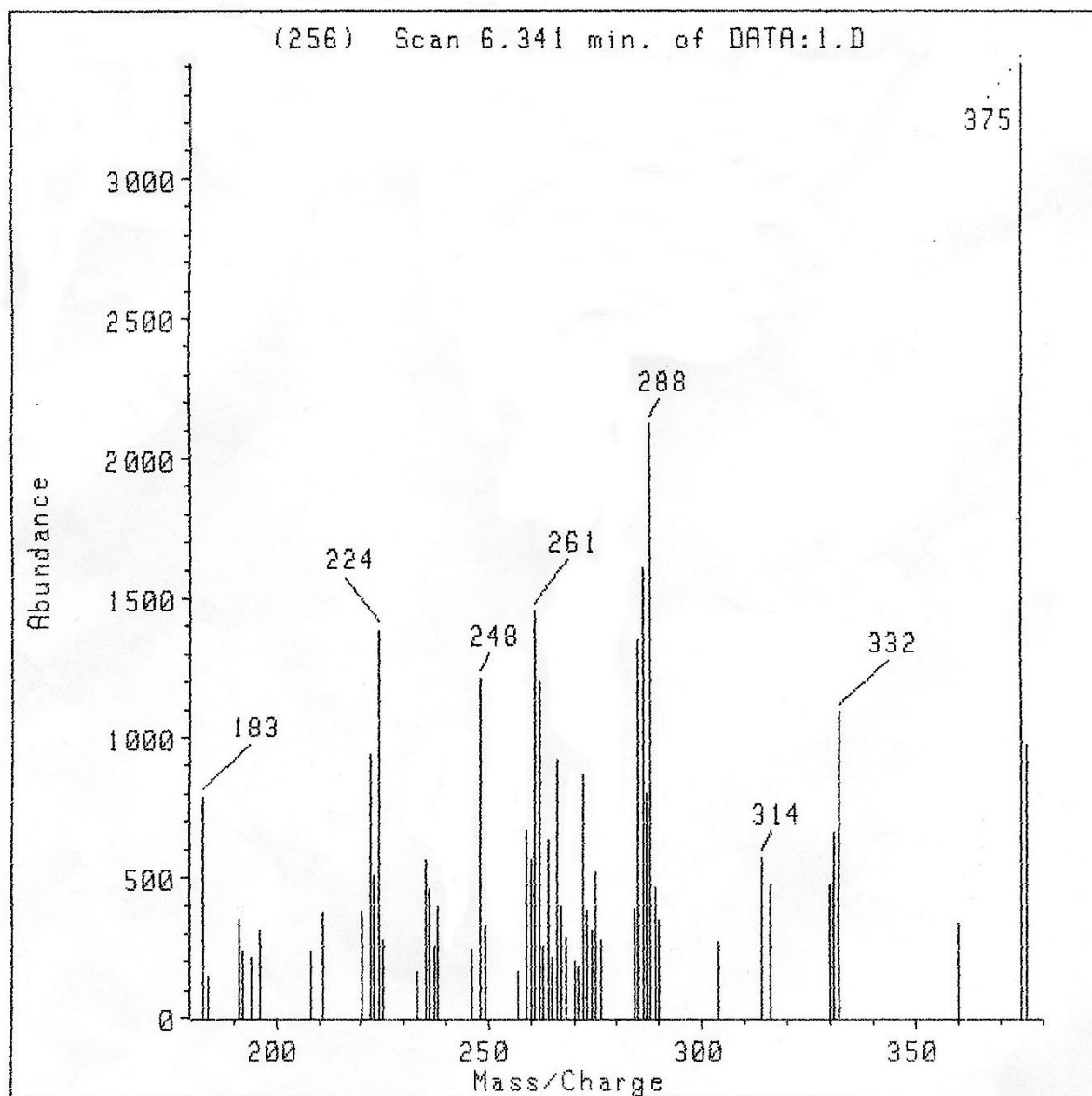
**Fig. 5. Mass spectrum of Fluvastatin Sodium acid degradate.**
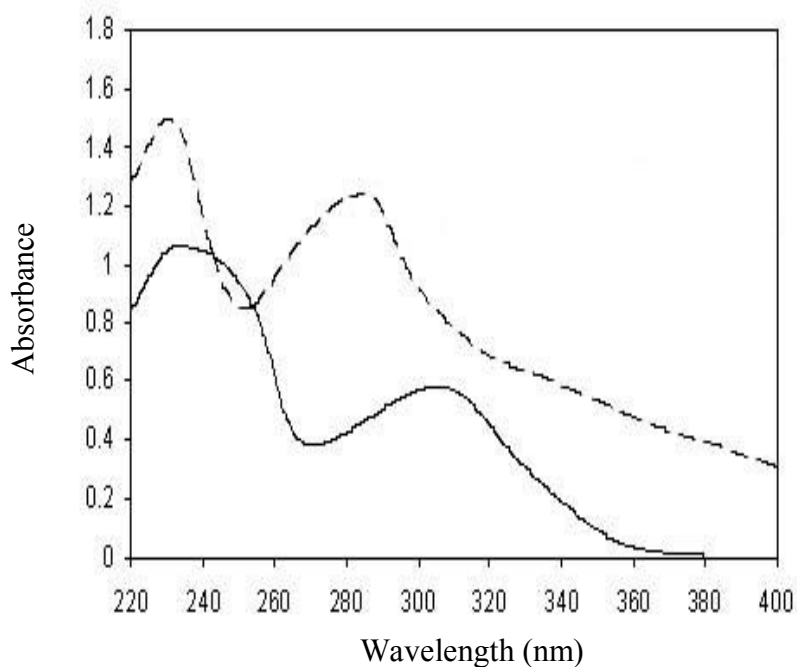
**Fig. 6. Zero-order absorption spectra of 20 μg.mL$^{-1}$ of Fluvastatin Sodium standard solution (—) and 20 μg.mL$^{-1}$ of its acid degradate solution (---) in methanol.**
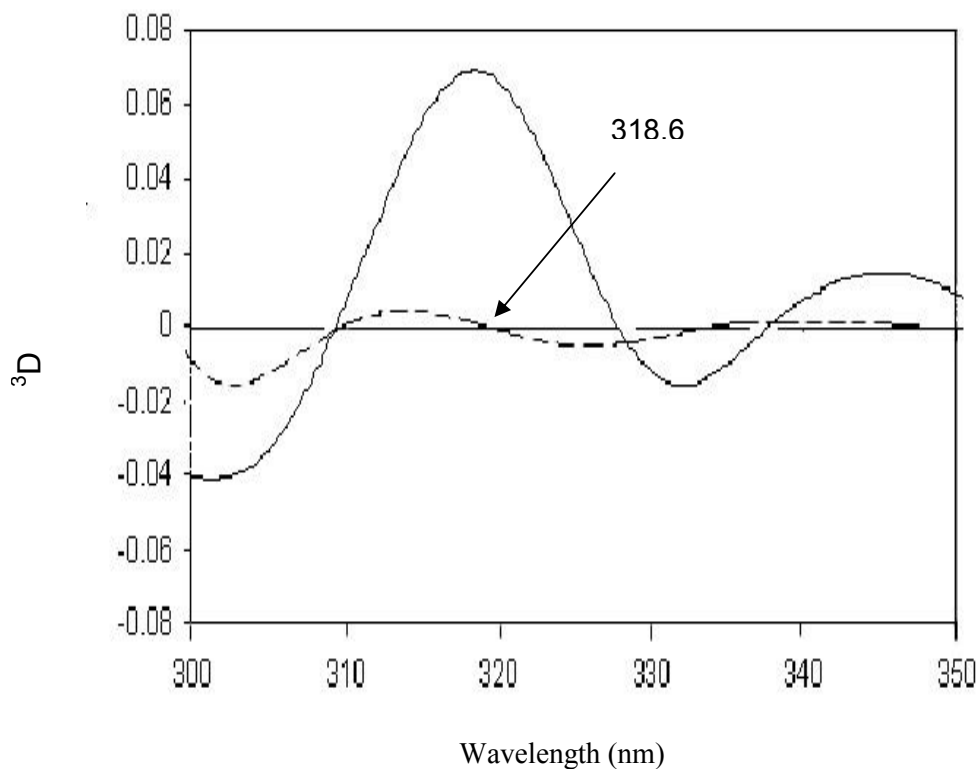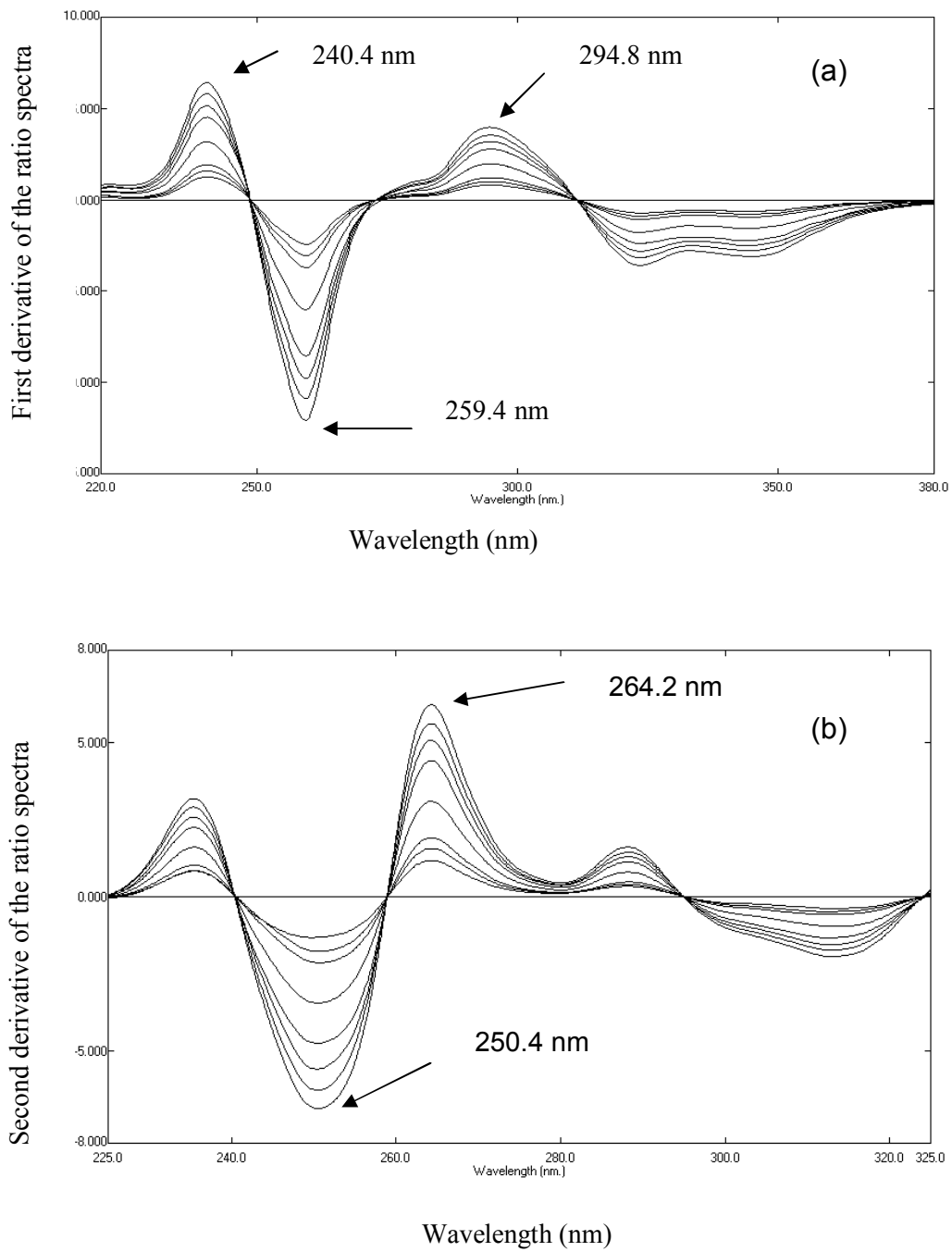


**Fig. 7. Third derivative absorption spectra of 20 μg.mL$^{-1}$ of Fluvastatin Sodium standard solution (—) and 20 μg.mL$^{-1}$ of its acid degradate solution (---) in methanol.**

**Fig. 8. First derivative of the ratio spectra (a) and second derivative of the ratio spectra (b) for different concentrations of Fluvastatin Sodium (4, 5, 6, 10, 14, 16, 18, 20 µg.mL$^{-1}$) using normalized spectrum of the acid degradate as divisor.**
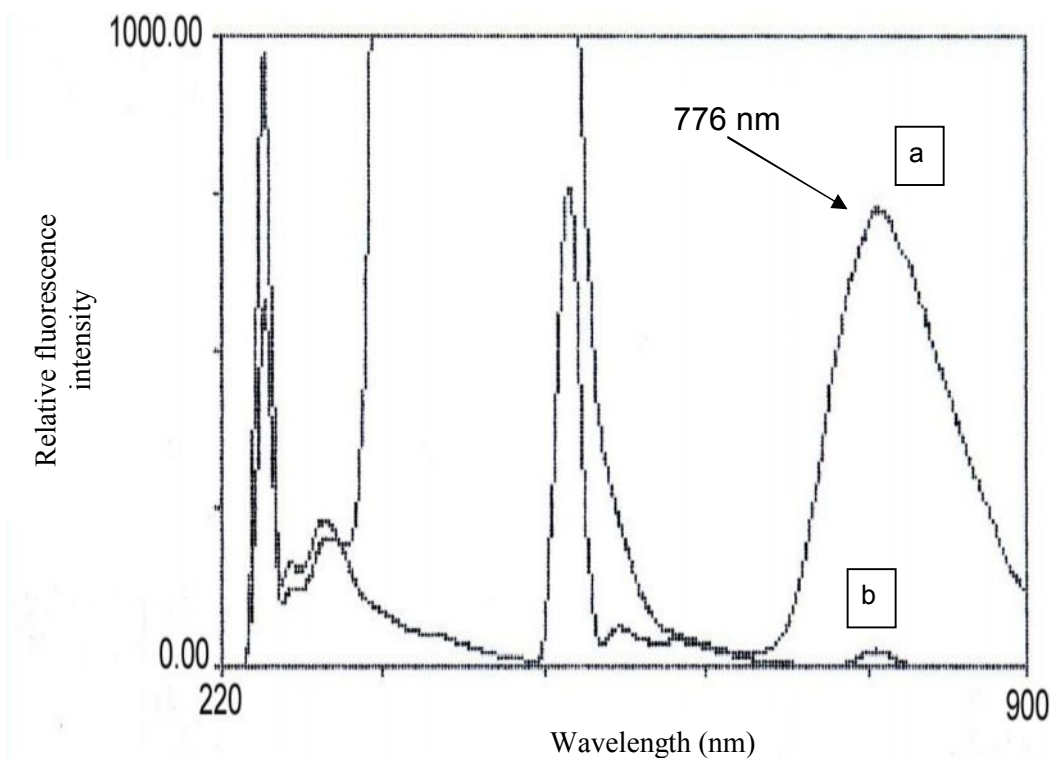
**Fig. 9. Excitation and emission spectra of 8 µg.mL$^{-1}$ of Fluvastatin Sodium in ethanol (a) and ethanol as blank (b).**
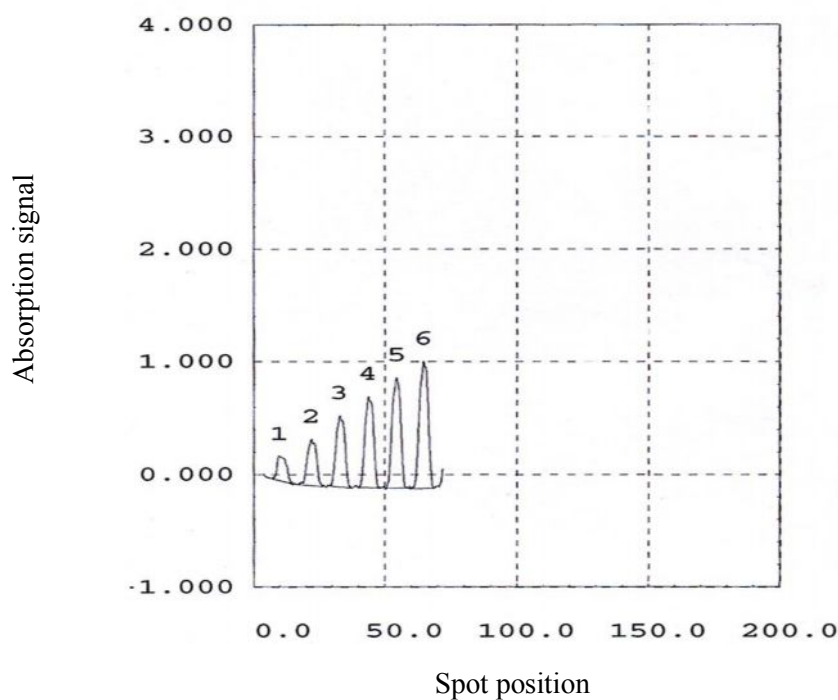


**Fig. 10. Scanning profile of the TLC chromatogram of Fluvastatin Sodium in the concentration range   1 – 10 µg/spot (Rf = 0.6) using  chloroform : hexane : methanol : glacial acetic acid (5 : 5 : 1 : 1, v/v/v/v) as developing system, at 304 nm.**
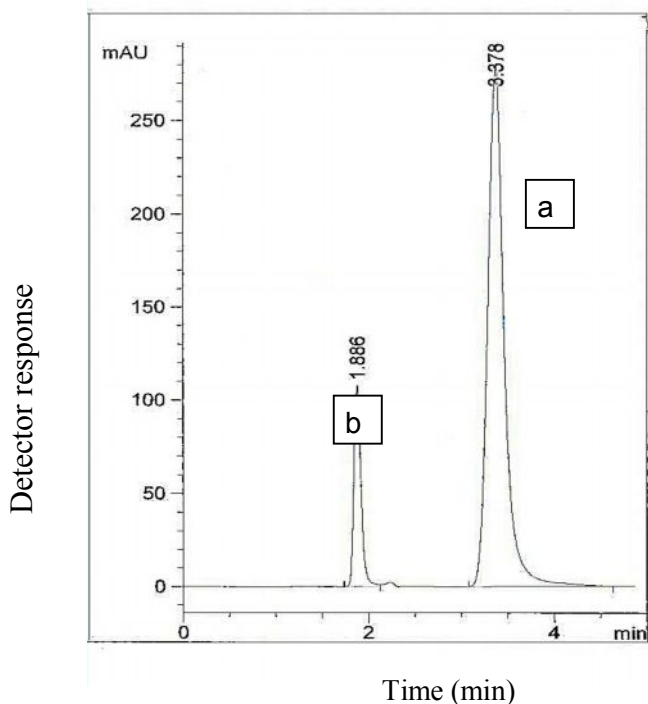
**Fig. 11. HPLC chromatogram of 50 µg.mL$^{-1}$Fluvastatin Sodium standard solution (a) and 40 µg.mL$^{-1}$ Sodium Benzoate solution as an IS (b) in mobile phase, experimental conditions (see section  2.1. ).**
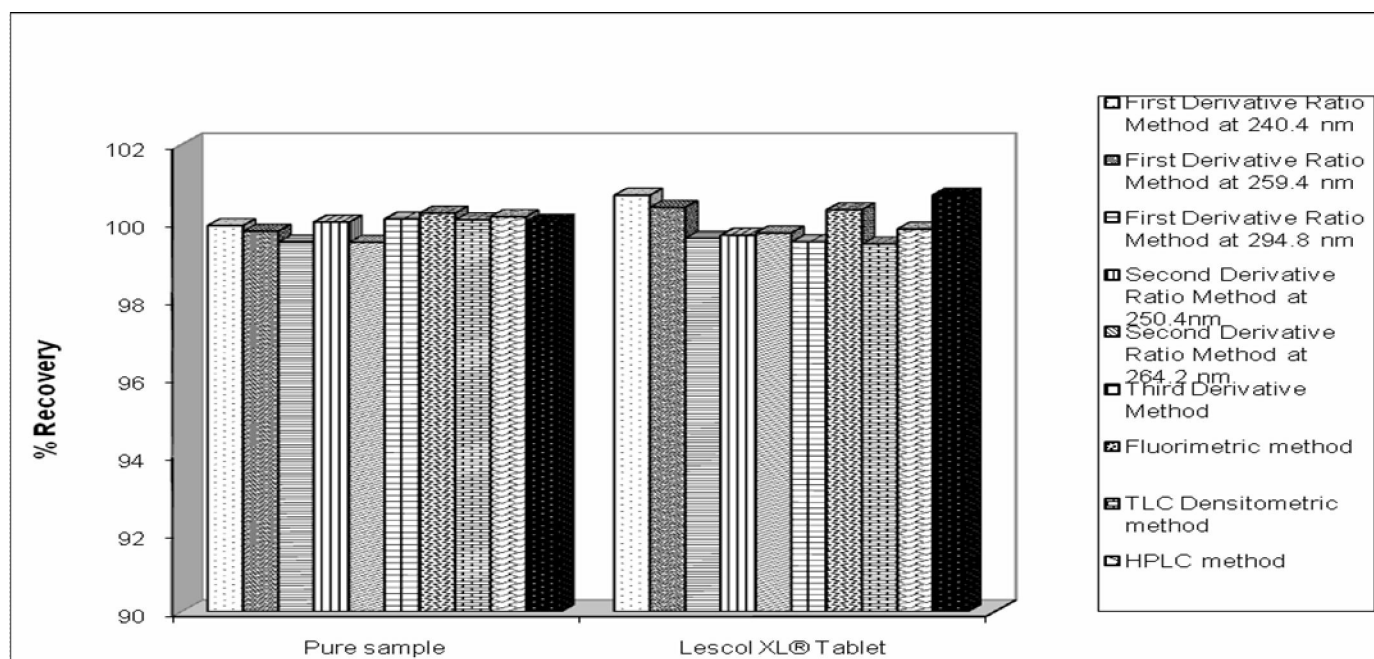


**Fig.  12. comparison between the results obtained by applying the proposed methods and the reference method for the analysis of Fluvastatin Sodium.**

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