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Stability study of simvastatin and pravastatin sodium under acidic hydrolytic conditions assessed by high performance liquid chromatography

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ABSTRACT

In this work, a liquid chromatography stability indicating method was developed and applied to study the hydrolytic behavior of simvastatin (I) and pravastatin sodium (II) in acid medium of different normalities at different temperatures. The developed chromatographic conditions were Zorbax C_8 (10µm,150mm × 4.6mm, i.d..) with particle size of 5 µm column, with a mobile phase consisting of methanol : de-ionized water (80 :20, v/v) ;and flow rate 1.5 ml / min. The method showed high sensitivity with good linearity over the concentration range of 2 to 25 µg / ml of both. The method is successfully applied to the analysis of pharmaceutical formulations containing (I) and (II) with excellent recovery. The kinetics investigation of the acidic hydrolysis of (I) and (II) are carried out in hydrochloric acid solutions of 1.0, 2.0 N and 4.0 N by monitoring the parent compound itself. The reaction order of (I) and (II) followed pseudo-first order kinetics. The activation energy could be estimated from the Arrhenius plot and it was found to be 11.82 and 11.22 Kcal/ mol. for simvastatin (I) and pravastatin sodium (II) respectively.

KEYWORDS

High performance liquid chromatography;
Simvastatin (I);
Pravastatin sodium (II);
Degradation;
Kinetics.

INTRODUCTION

Simvastatin and pravastatin sodium are cholesterol-lowering agent belonging to statin class, the most frequently prescribed and efficient drugs used to treat hypercholesterolemia and significantly reduce the morbidity and mortality associated with coronary heart disease[1]

Simvastatin is rapidly hydrolysed in vivo to its corresponding hydroxyl acid which is the active inhibitor of hydroxymethylglutaryl COA reductases, key enzymes in the production of cholestrol, while pravastatin sodium is administrated and absorbed in its hydroxyl acid form. The reductase inhibition is directly related to the structural similarity between the drug and the endogenous substrate; therefore, both the pharmacological and therapeutic activities of this drug have a close relationship with its structure. The basis of studies about the drug, stability from a structural point of view is the presence of reactive center i.e., ester moiety, that can be hydrolyzed.[2]

Investigation of the stability of drugs represents an important subject in drug quality evaluation. Many environmental conditions, such as heat, light, or the chemical susceptibility of substances to hydrolysis or oxida-

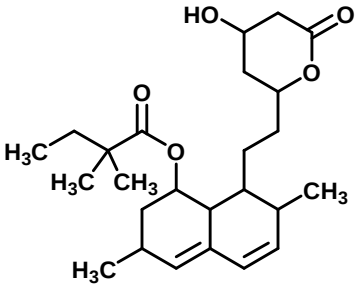
tion, can have a very serious role in pharmaceutical stability testing of drug substance can help to identify likely degradation products and give important information on the drug stability.

For this reason, this paper aimed to study the hydrolytic behavior of simvastatin and pravastatin sodium in presence of their acidic hydrolytic degradation products and develop and validate stability indicating high performance liquid chromatographic method used in monitoring the quality of pharmaceutical product.

Simvastatin

It is effective in reducing total and LDL-cholesterol as well as plasma triglycerides and apolipoprotein B. It inhibits the function of hydroxymethylglutaryl COA reductases, key enzymes in the production of holesterol[1]

Simvastatin[(1S,3R,7S,8S,8aR)-8-[2-[(2R,4R)-4-hydroxy-6-oxooxan-2yl]ethyl]-3,7-dimethyl 1,2,3,7,8,8a-hexahydronaphthalen-1-yl] 2,2-dimethylbutanoate (Figure 1) is a white crystalline powder, very soluble in methylene chloride and freely soluble in alcohol insoluble in water[3].



Molecular formula $C_{25}H_{38}O_5$
Molecular weight (418.6)

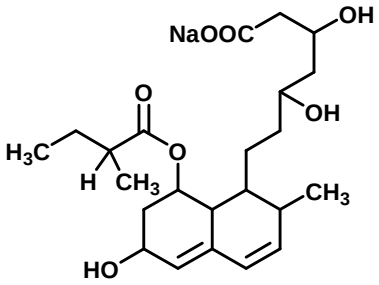
Figure 1 : Structure of simvastatin

Simvastatin have been determined by several methods including spectrophotometric methods[4,6], spectrofluorimetric method[6], electrochemical method[7,8], HPTLC[9] gas chromatography methods[10,11] and liquid chromatography methods[12-17] and HPLC[18-29]

Pravastatin sodium

It is one of the new classes of lipid lowering compounds; it affects blood cholesterol level by inhibiting cholesterogenesis in liver which results in increased expression of LDL receptor gene[30]

Pravastatin Sodium {1-naphthaleneheptanoic acid, 1, 2, 6, 7, 8,8a-hexahydro-β, δ, 6-trihydroxy-2-methyl-8-(2-methyl-1-oxobutoxy)-, mono sodium salt}, (Figure 2) is a white to yellowish white powder or crystalline powder hygroscopic freely soluble in water and methanol soluble in ethanol[31]



Molecular formula: $C_{23}H_{35}O_7$
Molecular weight: 446.5

Figure 2 : Structure of pravastatin sodium

The literature survey reveals several analytical methods for quantitative estimation of Pravastatin by various methods including spectrophotometric methods[32], Spectrofluorimetric method[6], electrochemical method[33-35], HPTLC[9], Gas chromatography methods[36-38] and HPLC[39-47]

The present work aimed to develop feasible, sensitive and specific analytical method for the analysis of simvastatin (I) and pravastatin sodium (II) respectively in presence of their degradation products. Adaptation of the proposed method for the analysis of the available dosage form including expired ones is also an important task in order to solve problems encountered in quality control. Moreover, kinetic studies and Accelerated stability experiments to predict expiry dates of pharmaceutical products necessitate such method

EXPERIMENTAL

Samples

Pure sample

a. Simvastatin powder was kindly supplied by Sigma pharmaceutical industries. Its purity was checked in our laboratory according to the official method of analysis[29] and was found to be 100.50 ± 0.751.
b. Pravastatin sodium powder was kindly supplied by Squibb. Its purity was checked in our laboratory according to the official method of analysis[47] and was found to be 100.07 ± 0.958.

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Pharmaceutical dosage forms

Simvastatin

1. Zocor tablets batch no.2534790 (10mg of simvastatin /tablet), 510304 (20 mg of simvastatin / tablet) and 610403 (40 mg of simvastatin / tablet) were purchased from the Egyptian market are manufactured by Global Napi pharmaceutical company. Egypt under license from Merck & Co Inc white house station. N.j U.S.P.
   Expired batches of Zocor tablets10 mg of simvastatin / table (batch no. A/2256610) (expired 6/2004) and (20 mg of simvastatin / tablet) batch no.A/2256210 (expired 6/2004)
2. Simvastat tablets batch no.114 (10mg of simvastatin /tablet) and batch no.122 (20 mg of simvastatin / tablet) are manufactured by Rexcl Egypt and were purchased from the Egyptian market.
3. Simvacor tablets batch no. 50563 (20 mg of simvastatin/tablet), 50567 (40mg of simvastatin/tablet) and 50570 (80 mg of simvastatin/tablet) are manufactured by Sigma and were purchased from the Egyptian market.

Pravastatin sodium

- Lipostate tablets batch no.18452/95(10 mg of pravastatin sodium /tablet) 17563/93 (20 mg of pravastatin sodium / tablet) and 22341/202 (40 mg of pravastatin sodium / tablet) were purchased from the Egyptian market are manufactured by Squibb pharmaceutical company Egypt.
- Expired batches of Lipostate tablets (batch no. B10539 (10mg of pravastatin sodium / tablet) (expired 10/2004) and batch no B10549 (20 mg of pravastatin sodium / tablet) (expired 10/2004).

Chemical and reagents

All chemicals used were of analytical grade, and de-ionized water for HPLC. Hydrochloric acid, methanol (HPLC grade), toluene, ethyl acetate, glacial acetic acid and sodium hydroxide were purchased from El Nasr Pharmaceutical Chemical Co. (Adwic).and E. Merck Darmstadt -Germany

Standard solutions

- Simvastatin standard solution (1 mg / ml) in methanol
- Pravastatin sodium standard solution (1 mg / ml) in methanol
- Drug degradation product standard solution (1 mg / ml): Accurate weight of SIM or PRA (50 mg) was introduced into a 50 ml round bottom flask, 50 ml of hydrochloric acid was added and the solution was refluxed at100°C for 30 min. The solution was neutralized using 4 N sodium hydroxide. The degradation product solution was applied on TLC plates in bands and the plates were developed in chromatographic tanks previously saturated with the developing system, toluene: acetone (70:30, v/v) in case of SIM, methanol: ethyl acetate: glacial acetic acid (68: 40: 28: 0.7, by volume) in case of PRA. The plates were air dried and visualized under UV light 254 nm.
  The bands of the degradation products were scraped extracted with methanol three times each with 30 ml portion and filtered. The combined methanolic extract was used to produce a concentration of 2 mg/ml of degradation products. All solutions were freshly prepared on the day of analysis and stored in a refrigerator to be used within 24 h.

Apparatus and chromatographic condition

- Precoated TLC-plates, silica gel 60 F_{254}(20 cm × 20 cm, 0.25 mm), E. Merck (Darmstadt-Germany)
- IR Spectrophotometer: Shimadzu 435(Kyoto, Japan), sampling was undertaken as potassium bromide discs
- Gas chromatograph coupled to a mass spectrophotometer GC–MS-QB1000EX, Finnigan Nat (USA)
- pH-meter, DigitalpH/MV/TEMP/ATC meter, Jenco Model 5005 (USA).
- Liquid chromatograph consisted of an isocratic pump (Agilent Model G1310A), an ultraviolet variable wavelength detector (ModelG1314A, Agilent 1100 Series), a Rheodyne injector (Model 7725 I, Rohnert Park, CA, USA) equipped with 20 µ L injector loop, Agilent (USA). Stationary phase; a 150 mm × 4.6 mm i.d. A Zorbax Eclipase × DB. C_8 analytical column, Alltech (USA). Mobile phase methanol: water (80:20,v/v); isocratically at 1 ml/min. The mobile phase was filtered through a 0.45 µ m Millipore membrane filter and was degassed

for 15 min in an ultrasonic bath prior to use UV-detection was done at 238 nm. The samples were filtered also through a 0.45 μ m membrane filter, and were injected by the aid of a 25μl Hamilton® analytical syringe.

Procedures

Preparation of degradation product

Degradation product of simvastatin

Accelerated acid-degradation was performed by dissolving 50 mg of pure SIM powder in 50 ml of 4 N hydrochloric acid. The solution was refluxed at100°C for 30 minutes. Complete degradation was achieved; as investigated by thin layer chromatography using toluene: acetone (70:30v/v) as a developing solvent and visualized under UV light at 254 nm.. The acid degraded sample was neutralized by 4 N sodium hydroxide (to pH 7.0). The solution was evaporated under vacuum nearly to dryness then re-crystallized from methanol. The obtained degradation product was characterized by UV-spectroscopy, TLChromatography, and GC/MS- and IR-spectrometry.

Degradation product of pravastatin sodium

Follow the same procedures as described under *2.5.1.a* using developing solvent consisting of toluene + methanol +ethyl acetate + glacial acetic acid (68 : 40: 28: 0.7 by volume).

Linearity

Transfer accurately aliquot volumes [0.02-0.25ml] of simvastatin and pravastatin sodium stock solution (1mg/ml in methanol) into two separate series of 10-ml volumetric flask, and complete the content of each flask to volume with methanol to get concentrations 2 – 25 μg / ml of simvastatin and pravastatin sodium. The samples were then chromatographed using the following chromatographic conditions. Stationary phase; a 150 mm × 4.6 mm, i.d. Zorbax C_8 analytical column, Alltech (USA), mobile phase methanol: water (80:20,v/v). The mobile phase was filtered through a 0.45 μm Millipore membrane filter and was degassed for about 15 min in an ultrasonic bath prior to use, flow rate; 1.5 ml/ min {isocratically at ambient temperature (≈25 °C)},with UV-detection at 238 nm. The samples were filtered also through a 0.45 μm membrane filter, and 20μl were injected by the aid of a 25μl Hamilton® analytical syringe. To reach good equilibrium, the analysis was usually performed after passing ˜50–60 ml of the mobile phase, just for conditioning and pre-washing of the stationary phase. Plot the relative peak area ratios (peak area of simvastatin or pravastatin sodium, to that of external standard) were then plotted *versus* the corresponding concentrations of simvastatin or pravastatin sodium in $\mu g.ml^{-1}$ to get the calibration graph and to compute the corresponding regression equations (1) and (2). Concentrations of unknown samples of simvastatin (I) or pravastatin sodium(II) were determined using the obtained regression equation.

Analysis of laboratory prepared mixtures containing different ratios of simvastatin or pravastatin sodium and its degradation product using the suggested method

Mix aliquots of intact drug and the degraded drug to prepare different mixtures containing 10–90% (w/w) of the degradation product, and proceed as mentioned under linearity. Calculate the concentrations of the drugs from the corresponding regression equations.

Assay of pharmaceutical formulations

Tablets were grinded, dissolved in 50 ml methanol, filtered then diluted with methanol to obtain the appropriate concentrations and chromatographed as under the linearity

Kinetic studies

For studying the kinetic order of the reaction for simvastatin and pravastatin sodium

Into two separately 50 ml volumetric flasks, accurately weighed 30 mg of simvastatin (I) or pravastatin sodium (II) in 50 ml 4.0N hydrochloric acid in screw-capped container. The solutions were refluxed in a thermostatically controlled water bath at 90°C for 30 minutes. At specified time intervals (5 minutes), 1.0 ml was withdrawn from the container, neutralize with 1 ml 4.0 N sodium hydroxide and a solution of concentration ($24\mu g.ml^{-1}$) was prepared. The degradation rate kinetics was determined using the proposed method by plotting log of concentration of drug remaining *versus* time. Each experiment was done in triplicate and average values were taken for the analysis.

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For studying the effect of hydrochloric acid concentration and the temperature on the reaction rate

The stability of simvastatin (I) or pravastatin sodium (II) in 1.0, 2.0, and 4.0 HCl, in a thermostatically controlled water bath at 60 °C, 70 °C, 80 °C and 90 °C for 30 minutes was studied. The degradation rate kinetics was determined by plotting log of concentration of drug remaining versus time for different normalities of HCl. Each experiment was done in triplicate (analysis by HPLC method) and average values were taken for the analysis. Plot the Arrhenius plot for the effect of temperature on the rate of hydrolysis and calculate the rate constant and $t_{1/2}$

RESULTS AND DISCUSSION

Complete degradation of simvastatin and pravastatin sodium was achieved by refluxing with 4N hydrochloric acid for 30 min. On the other hand, the degradation products of them were isolated using preparative TLC plates and characterized by UV-spectroscopy, GC/MS-and IR-spectrometry.

Degradation pathway

Degradation of simvastatin

Degradation of simvastatin(I) in 4N HCl, TLC monitoring of the drug degradation toluene: acetone (70:30v/v) as a was done on thin layer plates of silica gel F_{254}using developing solvent. The developed plates were visualized under short UV-lamp and. The acidic degradation product (R_f value = 0.89) could be separated elegantly from the intact drug (R_f value = 0.59)

Simvastatin and its acidic degradation product have different UV spectrum due to cleavage of the ester group followed by dehydration[48]

The GC/MS-chart, the parent peak was identified at m/z 284 (mol. wt. of degradation product $C_{19}H_{24}O_2$). (Figure 3)

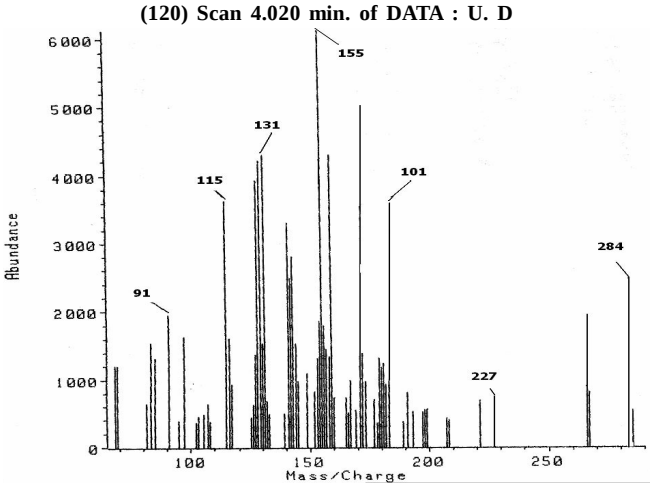
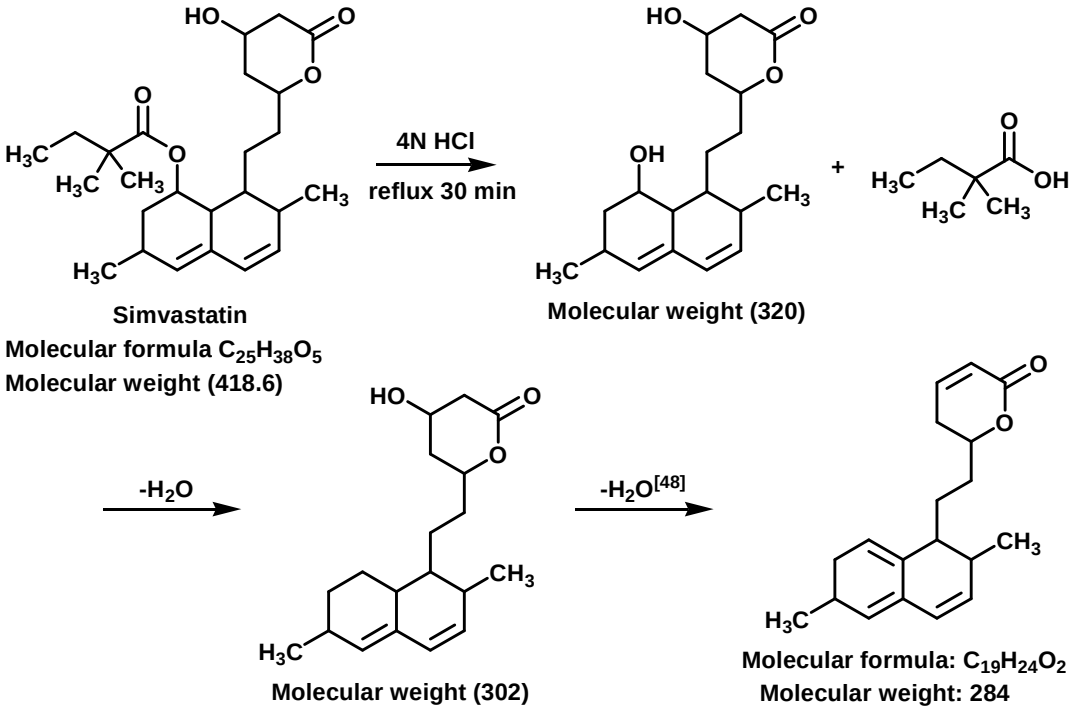


Figure 3 : Mass spectrum of degradation product of simvastatin



Proposed scheme for preparing the degradation product of simvastatin

This was assessed with IR. The assignments of the degradation product was based on comparison of the IR spectral data of the purified specimen, separated from the degradation reaction, with those of the intact compound as summarized in TABLE 1.

TABLE 1 : Assignment of IR characteristic band of intact simvastatin and its degradation product

Band or peak cm^{-1}	Possible Assignment[3,48]	Intact	Acid-induced degradation product
-Broad band at 3443.8	OH stretch	present	absent
-Band at 2800-2900	C-H aliphatic	present	present
-Band at 1718-1721	Carbonyl (Lactone, ester or both)	present	present
-Band at 1260	Lactone	present	present
-Band at 1056	Ester	present	absent

Degradation of pravastatin sodium

Degradation of pravastatin sodium (II) in 4N HCl, TLC monitoring of the drug degradation was done on thin layer plates of silica gel F_{254} using toluene + methanol +ethyl acetate + glacial acetic acid (68 : 40: 28: 0.7 by volume) as a developing solvent. The developed plates were visualized under short UV-lamp. The acidic degradation product (R_f value = 0.81) could be separated elegantly from the intact drug (R_f value = 0.45).. No other degradation products could be observed under all the different degradation conditions. A bathochromic shift at 265 nm which is characteristic λ_{max} of naphthalene ring containing compounds was observed during degradation of pravastatin sodium.

The GC/MS-chart, the parent peak was identified at m/z 266 (mol. w. of degradation product ($C_{18}H_{18}O_2$). (Figure 4)

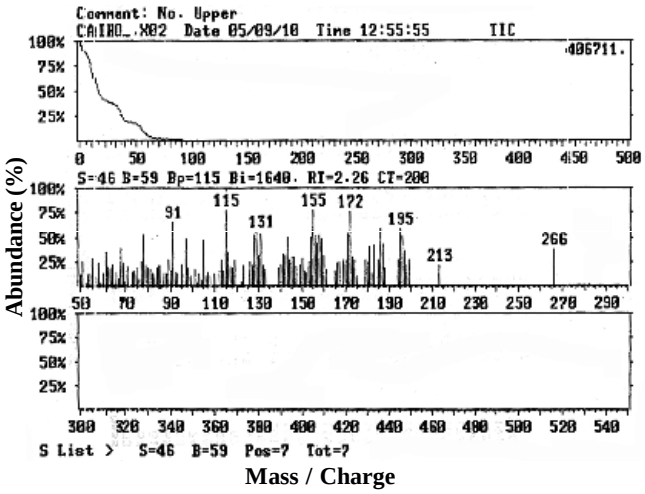
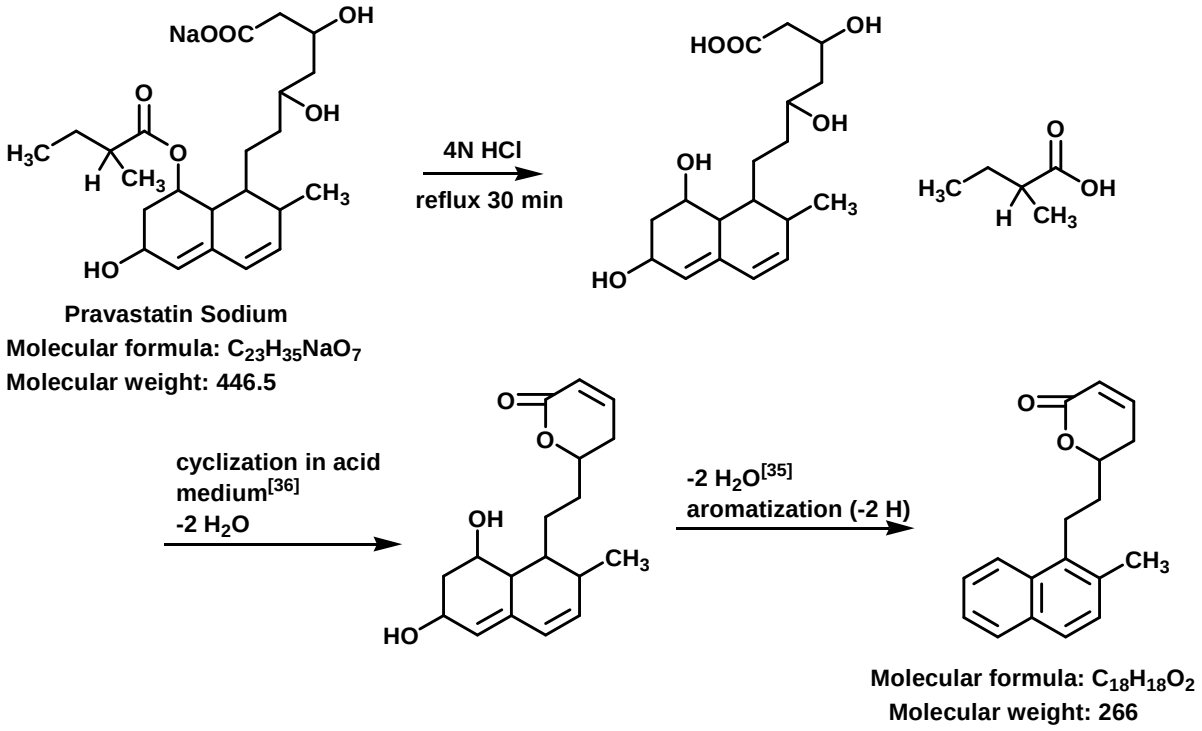


Figure 4 : G.C Mass spectrum of degradation product of pravastatin sodium

The expected degradation product of pravastatin sodium has a lactone ring as the carboxylic acids whose molecules have a hydroxyl group on a γ or δ carbon



Proposed scheme for preparing the degradation product of pravastatin sodium

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undergo an intermolecular esterification to give cyclic esters known as γ or δ lactones. The reaction is acid catalyzed[49]. This was assessed with IR. The assignments of the degradation product were based on comparison of the IR spectral data of the purified specimen, separated from the degradation reaction, with those of the intact compound as summarized in TABLE 1.

TABLE 2 : Assignment of IR characteristic band of intact pravastatin sodium and its degradation product

Band or peak cm^{-1}	Possible Assignment[3,48]	Intact	Acid-induced degradation product
-Broad band at 3357.6	OH stretch	present	absent
-Band at 3056.5 and 3025.8	C-H aromatic stretch	absent	present
-Band at 2800-2900	C-H aliphatic	present	present
-Sharp band at 1729.4	Ester	present	absent
-Band at 1579.9	Carboxylate anion COO-	present	absent
-Band at 1720	Lactone	absent	present
-Band at 1250	Lactone	absent	present

High performance liquid chromatographic analysis

To date, no LC stability indicating analytical method has been described in the literature, and no previous systematic studies focused on simvastatin and pravastatin sodium acidic degradation have been performed. The suggested mechanism of them shows completely pharmacological inactive degradation products due to their lack of ester moiety. For this reason, this paper aimed to study the hydrolytic behavior of simvastatin and pravastatin sodium in presence of their acidic hydrolytic degradation products

Simple isocratic high-performance liquid chromatographic methods are described for the determination of simvastatin (I) and pravastatin sodium (II) in presence of its acidic degradation product without prior separation. To optimize the HPLC assay parameters, the type of column and its dimension, mobile phase condition, the choice of wavelength of detection were investigated. Different type of stationary phase C_8, C_{18} and a Zorbax ODS column with different dimensions and particles size were used. It was found that the Zorbax C_8 (10 µl, 150mm cm × 4.6 mm i.d.) with particle size of 5 µm gave the most suitable resolution for both simvastatin (I) and pravastatin sodium (II). The peak shape improved dramatically with an increase in the percentage of methanol in the mobile phase. Satisfactory separation was performed with a mobile phase consisting of methanol: deionized water (80:20, v/v) for both, with a retention time of retention time of 4.325 ± 0.03 min. for simvastatin and 5.040 ± 0.02 min for its acid-induced degradation product and with a retention time of 1.331 ± 0.04 min. for pravastatin sodium and 2.429 ± 0.03 min for its acid-induced degradation product. Figures 5 and 6. System suitability was checked by calculating the capacity factor, tailing factor, column efficiency and the selectivity factor (resolution) [TABLE 13].

A linear relationship was obtained between the simvastatin (I) and pravastatin sodium (II) the relative peak area at the selected wavelength (238 nm) and the corresponding concentrations of (I, II) in the range of 2 – 25 µg/ ml.

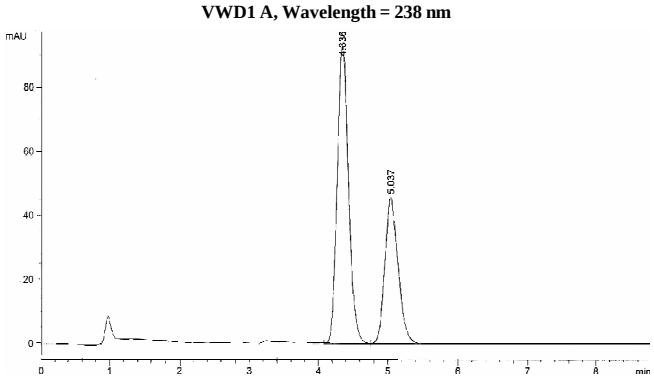

Figure 5 : Liquid chromatographic separation of simvastatin (I) (23 µg /ml) and its degradation product (2 µg /ml) using chromatographic conditions described in the text.

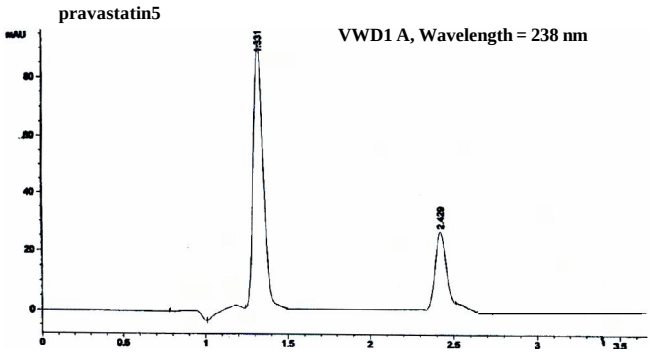

Figure 6 : Liquid chromatographic separation of pravastatin sodium (20 µg ml^{-1}) and its degradation product (5 µg ml^{-1}) using chromatographic conditions described in the text

The regression equation was computed and found to be:

A = 0.2068 C + 0.0683 r = 0.9997 for simvastatin
A = 0.2076 C + 0.0362 r = 0.9999 for pravastatin sodium)

Where A is the relative peak area (peak area of simvastatin and pravastatin sodium to that of external

standard), C is the concentration of the drug in μg/ml and r is the correlation coefficient.

Method validation

The selectivity and specificity of the proposed method was proved by the analysis of laboratory prepared mixtures containing different ratios of the drug and its degradation product and it was found to be valid until 92 % of degradation product. TABLE 3 for both I and II

TABLE 3 : Determination of simvastatin (I) and pravastatin sodium (II) in their laboratory prepared mixtures by the proposed method

Concentration ($\mu g.ml^{-1}$)		Percentage %		HPLC Method	
Drug (I)	Acid-induced degradation product	Drug (II)	Acid-induced degradation product	Recovery % (I)	Recovery % (II)
23.0	2.0	92	8	98.56	100.26
21.0	4.0	84	16	99.32	101.11
17.0	8.0	68	32	100.12	100.34
12.5	12.5	50	50	99.78	99.65
8.0	17.0	68	68	100.01	99.21
4.0	21.0	84	84	99.37	98.99
2.0	23.0	92	92	101.01	101.02
Mean				99.73	99.91
S.D				± 0.773	± 0.791
RSD				0.775	0.792

To ascertain the accuracy of the proposed procedure, it was successfully applied for the determination of simvastatin in its pharmaceutical dosage forms and pravastatin sodium in Lipostate tablets TABLES 4, 5 and its validity was assessed by applying the standard addition technique. The small relative standard deviations indicate that the method is accurate. TABLES 6, 7.

The results obtained for the analysis of simvastatin and pravastatin sodium in the pure powdered form were statistically compared with those obtained by applying the official methods[29,47] and significant difference was not observed. TABLES 8, 9

The precision of the suggested method was also expressed in terms of relative standard deviation of the inter-day and intra-day analysis. The method was checked for its robustness by minor changes in assay conditions and it proved to be robust. Changes in instruments or personnel did not alter the results, which

TABLE 4 : Determination of simvastatin in its pharmaceutical dosage forms by the proposed method

Pharmaceutical dosage form	HPLC method	Official method*[29]
	Found % ± S.D**	Found % ± S.D**
1-Zocor tablets		
Batch number		
-2534790 (10 mg)	99.28 ± 1.184	100.71 ± 0.681
-510304 (20 mg)	99.18 ± 1.282	100.23 ± 0.672
-610403 (40 mg)	99.34 ± 1.023	100.41 ± 0.594
Batch number of expired tablets 6/2004		
-A/2256610 (10 mg)	89.16 ± 0.824	90.27 ± 0.824
-A/2256210 (20 mg)	87.93 ± 0.712	90.52 ± 0.873
2-Simvastat tablets		
Batch number		
-114 (10 mg)	100.11 ± 0.911	100.26 ± 0.453
-122 (20 mg)	99.55 ± 0.961	100.91 ± 0.741
3-Simvacor tablets		
Batch number		
-50563 (20 mg)	98.55 ± 0.421	100.11 ± 0.261
-50567 (40 mg)	99.83 ± 0.781	100.83 ± 0.931
-50570 (80 mg)	99.29 ± 0.631	100.24 ± 0.229

***Official method (USP 2006) HPLC using C18 column and Acetonitrile:buffer phosphate (pH 4.5) (650:350 v/v) as a mobile phase**

****Average of six different experiments.**

TABLE 5 : Determination of pravastatin sodium in its pharmaceutical dosage forms by the proposed method

Lipostate tablets	HPLC method	Official method*[47]
	Found % ± S.D**	Found % ± S.D**
Batch number		
-18452/95 (10 mg)	100.40 ± 1.352	100.23 ± 0.674
-17563/93 (20 mg)	100.33 ± 1.121	100.11 ± 0.682
-22341/202 (40 mg)	100.29 ± 0.823	100.03 ± 1.021
Batch number of : expired tablets10/2004		
-B10539 (10 mg)	92.35 ± 1.021	91.91 ± 1.023
-B10549 (20 mg)	91.71 ± 0.934	90.31 ± 0.674

***Official method (B.P 2005) using C18 column and glacial acetic acid : triethylamine : methanol : water (1:1:450:550 by volume) as a mobile phase.**

****Average of six different experiments**

TABLE 6 : Application of standard addition for the determination of simvastatin by the proposed HPLC method.

Zocor tablets (20mg)	Claimed amount taken (μg/ml)	Standard added (μg/ml)	Recovery % of added
Batch number 510304	6	4	99.77
		6	99.86
		8	100.50
		10	100.80
		12	100.90
Mean + S.D			100.37 ± 0.532
RSD			0.530

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TABLE 7 : Application of standard addition for the determination of pravastatin sodium by the proposed HPLC method

Lipostate tablets (20mg)	Claimed amount taken (µg/ml)	Standard added (µg/ml)	Recovery % of added
Batch number 17563 / 93	6	4	100.05
		6	101.76
		8	100.00
		10	99.52
		12	100.88
Mean + S.D			100.40 ± 0.864
RSD			0.861

TABLE 8 : Statistical comparison for the results obtained by the proposed method and the official method for the analysis of simvastatin in pure powdered form

Item	HPLC method	Official method*[29]
Mean	99.99	100.50
S.D	0.951	0.751
Variance	0.904	0.564
N	6	6
F test(5.05)	1.60	
Student's t-test(2.228)	2.134	

***Official method (U.S.P 2006) HPLC**
The figures in parenthesis are the corresponding tabulated values of F and t at (P = 0.05).

TABLE 9 : Statistical comparison for the results obtained by the proposed method and the official method for the analysis of pravastatin sodium in pure powdered form

Item	HPLC method	Official method*[47]
Mean	99.84	100.70
S.D	±1.183	±0.958
Variance	1.399	0.918
n	6	6
F test (5.05)	1.52	
Student's t-test (2.228)	1.353 (2.228)	

***Official method (B.P2005) HPLC.**
The figures in parenthesis are the corresponding tabulated values of F and t at (P = 0.05).

indicate the ruggedness of the proposed method. The obtained assay parameters and a validation sheet are presented in TABLE 10.

Kinetics of the degradation

A linear relationship was obtained by plotting the log concentrations of the remaining (I) and (II) against time Figures 7 and 8. Since the hydrolysis was performed in a large excess of hydrochloric acid (4.0 N) for simvastatin and pravastatin sodium, therefore it follows a pseudo-first order reaction rate which is the term used when two reactants are involved in the reaction but one of them is in such a large excess (HCl) that any change in its concentration is negligible compared with the change in concentration of the other reactant (drug).

TABLE 10 : Assay validation sheet of the proposed method for the determination of simvastatin and pravastatin sodium

Item	HPLC method	
	Simvastatin	Pravastatin Sodium
- Linearity		
Range (µg/ml)	2-25	2-25
Accuracy		
Mean	99.99	99.84
S.D	0.951	1.183
RSD	0.951	1.185
-Regression equation		
Slope (b)	0.2068	0.2076
Intercept	0.0683	0.0362
Correlation coefficient	0.9997	0.9999
R.S.D %*[a]	0.451	0.794
R.S.D%*[b]	0.632	0.844

***an intraday (n = 6) and *b the interday (n = 6) relative standard deviations of samples concentration (5, 10 and 15µg/ml) for simvastatin and (5, 10 and 25µg/ml) for pravastatin sodium using the proposed HPLC methods.**

Different parameters that affect the rate of the reaction were studied. The effect of temperature was studied by conducting the reaction at different temperatures using different concentrations of the acidic solution Figures 9-14. At each temperature the rate constant and $t_{1/2}$ were calculated then the log of the rate constant was plotted against the reciprocal of the temperature in Kelvin units (Arrhenius plot Figures 15, 16 to demonstrate the effect of temperature on the rate constant. It was concluded that as the temperature increased the rate of hydrolysis increased with a decrease in the $t_{1/2}$ TABLES 7, 8. Also the energy of activation was determined by calculating the rate constant from the following equation[50].

$$\text{Log}\frac{K2}{K1} = \frac{Ea}{2.303\,R}\left(\frac{T_2 - T_1}{T_1 T_2}\right)$$

Where "Ea" is the activation energy, "T_1" and "T_2" are the two temperature degrees in Kelvin, "R" is the gas constant (2. Cal. / K°.mol.), and "K_1" and "K_2" are the

rate constant at the two temperature degrees used.

The calculated "Ea" was found to be (11.82, 11.22) kilo calories mol^{-1} for (I) and (II) respectively which was comparatively a low value for esters, suggesting the instability of both in acidic medium.

Another factor that affects the rate of the reaction is the alkaline strength of HCL, thus different normalities of HCl solutions were used to study the hydrolysis

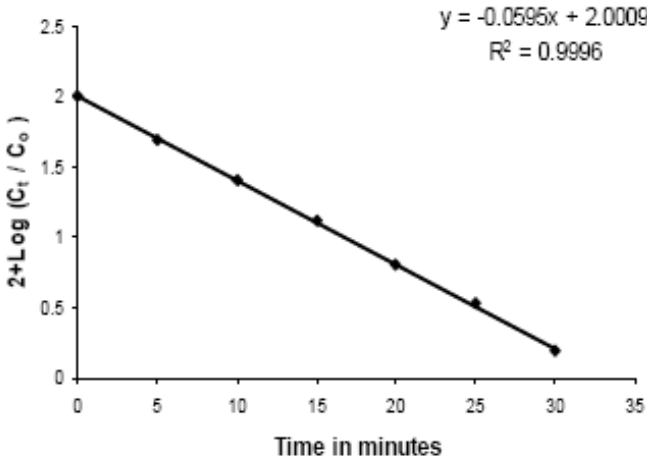

Figure 7 : First order plot of the hydrolysis of simvastatin (60 mg %) with 4.0N HCl at 90°C.

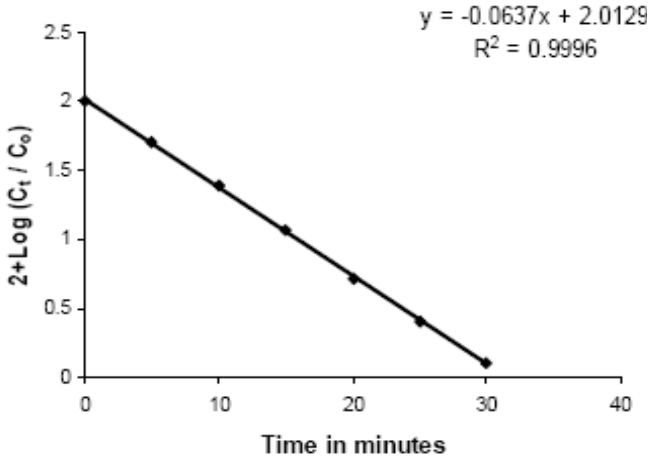

Figure 8 : First order plot of the hydrolysis of pravastatin sodium (60 mg %) with 4.0N HCl at 90°C.

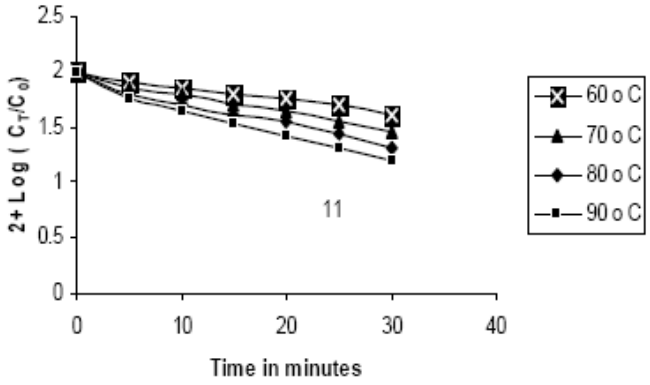

Figure 9 : First order plot of the hydrolysis of simvastatin (60mg %) with 1.0N HCl at different temperatures (60, 70, 80, 90°C).

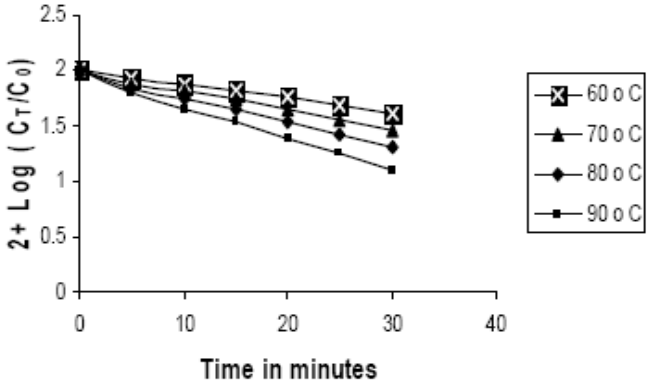

Figure 10 : First order of the hydrolysis of pravastatin sodium (60mg %) with 1.0 N HCl at different temperatures (60, 70, 80, 90°C).

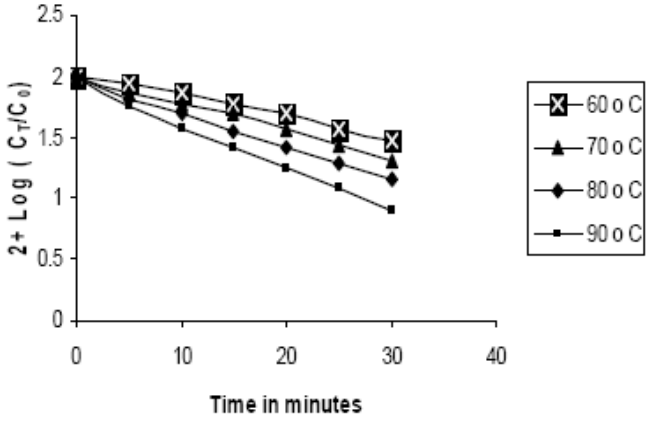

Figure 11 : First order plot of the hydrolysis of simvastatin (60mg %) with 2.0N HCl at different temperatures (60, 70, 80, 90°C).

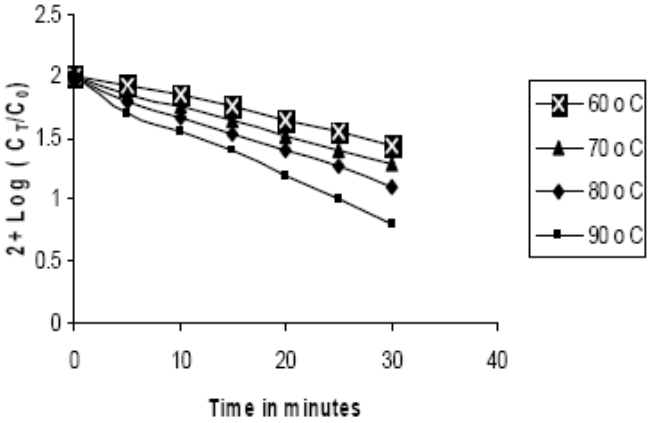

Figure 12 : First order of the hydrolysis of pravastatin sodium (60mg %) with 2.0N HCl at different temperatures (60, 70, 80, 90°C).

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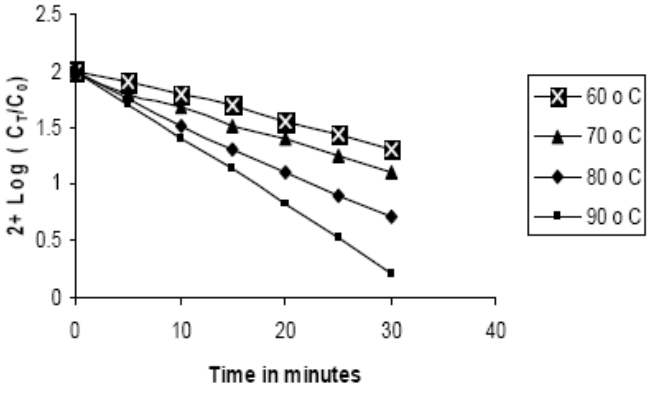

Figure 13 : First order of the hydrolysis of simvastatin (60mg %) with 4.0 N HCl at different temperatures (60, 70, 80, 90°C).

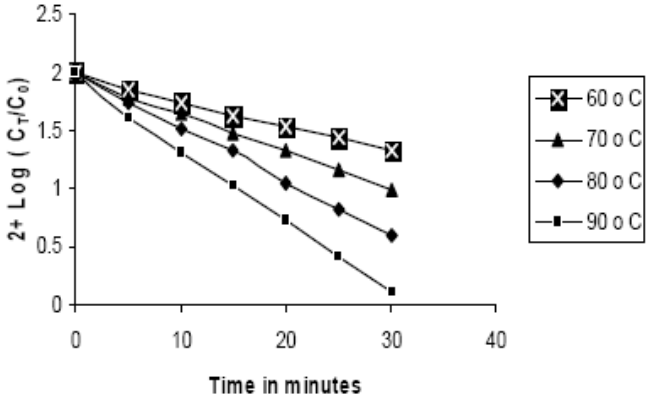

Figure 14 : First order of the hydrolysis of pravastatin sodium (60mg%) with 4.0N HCl at different temperatures (60, 70, 80, 90°C).

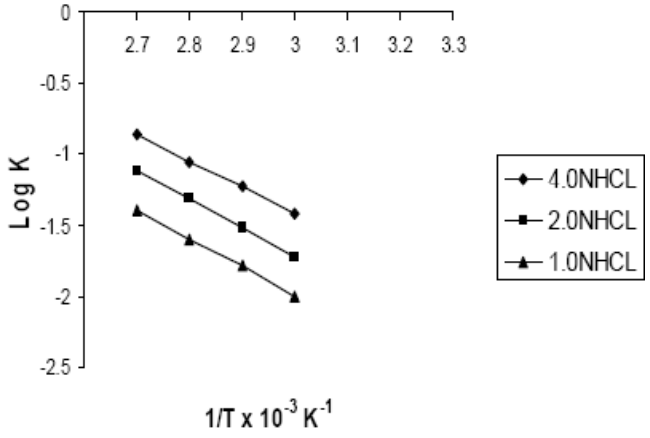

Figure 15 : Arrhenius plot for the hydrolysis of simvastatin (60mg %) with 1.0, 2.0 and 4.0N HC (K: Kelvin).

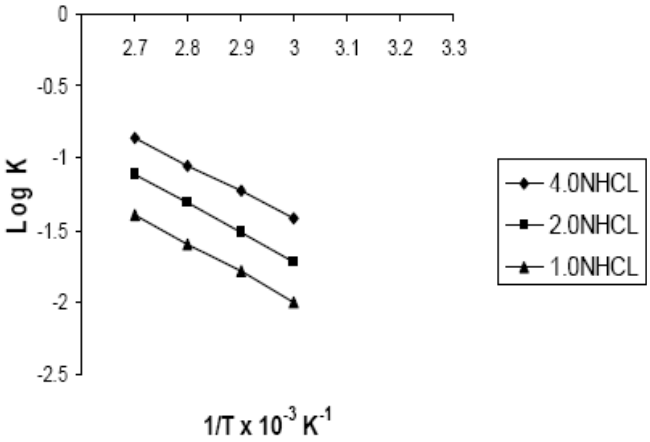

Figure 16 : Arrhenius plot for the hydrolysis of pravastatin sodium (60 mg %) with 1.0, 2.0 and 4.0N (K: Kelvin).

TABLE 11 : Parameters required for system suitability test of HPLC method

Parameter	Obtained value		Reference value[51,52]
Resolution (R)	1.402	1.996	R > 0.8
T (tailing factor)	SIM.: 1.0 Deg. Product 0.938	PRA.: 1.042 Deg. Product 0.071	T = 1 for a typical symmetric peak
α (relative retention time)	1.162	1.825	>1
K' (column capacity)	SIM. 3.336 Deg. Product: 4.037	PRA.: 0.331 Deg. Product: 1.429	1-10 acceptable
N (column efficiency)	SIM.: 835.595 Deg. Product: 2537.137	PRA.: 57.847 Deg. Product: 590.004	Increases with efficiency of the separation
HETP	SIM.: 0.018 Deg. Product: 0.006	PRA.: 0.259 Deg. Product: 0.025	The smaller the value, the higher the column efficiency

SIM.: Simvastatin
PRA.: Pravastatin sodium
Deg. product: Acid induced degradation product
Peak width in cm
Column length in cm

TABLE 12 : Kinetic data of simvastatin hydrolysis.

Normality of HCl	Temperature	K in min^{-1}	$t_{1/2}$ in min.
4.0 N HCl	90	0.137	5.1
	80	0.088	7.8
	70	0.059	11.8
	60	0.038	18.2
2.0 N HCl	90	0.076	9.1
	80	0.049	14.1
	70	0.030	23.1
	60	0.019	35.7
1.0 N HCl	90	0.039	17.8
	80	0.025	27.5
	70	0.016	43.3
	60	0.010	69.3

reaction. The rate of hydrolysis increase with increasing HCl concentration but to a minor effect compared to the effect of temperature Figures 7, 8, 9, 10, 11 and 12 and TABLES 7, 8.

TABLE 13 : Kinetic data of pravastatin sodium hydrolysis.

Normality of HCl	Temperature	K in min^{-1}	$t_{1/2}$ in min.
4.0 N HCl	90	0.147	4.7
	80	0.096	7.2
	70	0.062	11.2
	60	0.040	17.3
2.0 N HCl	90	0.078	8.9
	80	0.050	13.8
	70	0.032	21.7
	60	0.020	33.9
1.0 N HCl	90	0.042	16.5
	80	0.027	25.7
	70	0.017	40.8
	60	0.011	66.0

As a conclusion, the acidic hydrolysis of simvastatin (I) and pravastatin (II) were found to follow a pseudo first order reaction rate. Also the reaction rate increases with increasing in the temperature and the strength of the acidic solution

CONCLUSION

The proposed HPLC method provides a simple, sensitive, and selective and stability indicating method suitable for the quality control analysis of simvastatin (I) and pravastatin sodium (II) either in the pure powdered form or available pharmaceutical dosage forms.

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