# ASSESSMENT OF THE HYDROLYTIC DEGRADATION OF LOVASTATIN BY HPLC

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## ABSTRACT

In this work an HPLC stability-indicating method was developed and applied to study the hydrolytic behaviour of lovastatin in different simulated fluids. The selected chromatographic conditions were a C-18 column, acetonitrile/methanol/phosphate buffer solution pH 4 (32/33/35) as mobile phase, 45°C temperature column, flux of 1.5 mL/min and UV detection at 238 nm. The developed method exhibited an adequate repeatability and reproducibility (CV 0.057% and 0.73%, respectively) and a recovery higher than 98%. Furthermore, the detection and quantitation limits were $9.1\times10^{-7}$ M and $2.8\times10^{-6}$ M.

Lovastastin exhibited a pH-dependent degradation with an instantaneous hydrolysis in alkaline media at room temperature. One or two degradation products could be observed when lovastatin is hydrolyzed in alkaline or acid medium, respectively. The degradation products from lovastatin retain the UV-spectra of the parent drug, evidencing that the chromophore structure remains unaltered. Also, lovastatin hydrolysis in different media follows a pseudo first-order kinetic.

The rank-order for lovastatin stability in different media was: simulated gastric medium without pepsin > 0.06 M HCl > 0.1 M HCl > phosphate buffer pH 7.4 + sodium laurylsulphate > phosphate buffer pH 7.4.

**Key words:** lovastatin, HPLC, hydrolysis, stability, simulated fluids.

## INTRODUCTION

Lovastatin (Figure 1) is a drug that inhibits the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, enzyme that participates in the endogenous cholesterol synthesis, supporting its clinical use in the treatment of hypercholesterolemia [1, 2].

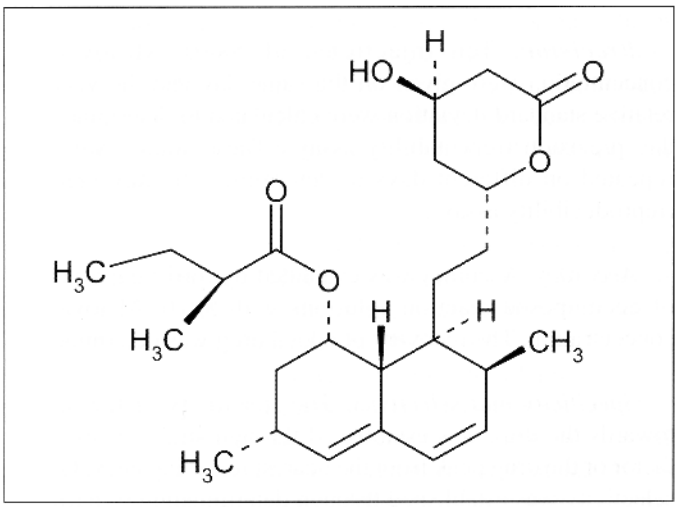


**Figure 1.** Chemical structure of lovastatin

The HMG-CoA reductase inhibition is directly related with the structural similitude between lovastatin and the endogenous substrate, HMG-CoA. Thus, both the pharmacological and therapeutic activities of this drug exhibit a close relationship with its chemical structure, which makes sense to carry out studies on its stability. From a structural point of view, lovastatin possess some chemically reactive centres, i.e., esters and lactones moieties, which can be hydrolysed.

The investigation of the stability of drugs represents an important issue in drug quality evaluation. Many environmental conditions such as heat, light, moisture as well as the chemical susceptibility of substances to hydrolysis or oxidation can play extremely serious role in pharmaceutical stability. A stress testing of drug substance can help to identify the likely degradation products and to provide important information on drug's inherent stability. Consecutively, it can be a fundamental contribution to development and validation of stability indicating analytical method used in monitoring of quality of pharmaceutical products [3,4]. Independent of the final dosage form, forced drug degradation by exposure of drug solution to acid or alkaline conditions is useful to predict the potential hydrolytic degradation products. Hydrolysis is one of the most common degradation chemical reactions. Since water, either as a solvent or in the form of the potential moisture in the air, contacts most

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pharmaceutical dosage forms to some degree; the potential for this degradation pathway exists for most drugs and excipients [5,6] .

Stability studies are designed to give an insight into the drug degradation mechanism, half-life (one-half the original concentration) and expiry dating ($t_{90}$, shelf-life) estimation. Shelf-life is defined as the time required for a drug to decompose to 90% of its initial concentration at a specific temperature, that is, 10% decomposition. The establishment of the prospective expiry date is of prime importance to drug product stability. Accelerated stability testing using the Arrhenius relationship is often employed for stability parameter identification. The well-known classical approach consists of sequential steps, that includes the determination of the kinetic constant $k_{pH,T}$ for the correct order of the degradation reaction. This is done through the functional relationship between drug content and time at several temperatures [7].

Lovastatin has been determined in biological fluids by high performance liquid chromatography-spectrophotometry [8-13], high performance liquid chromatography-tandem mass spectrometry [14], liquid chromatography-mass spectrometry-mass spectrometry [15,16], gas chromatography-mass spectrometry [17] and polarography [18]. In pharmaceutical dosage forms by micellar electrokinetic chromatography [19], supercritical fluid chromatography [20] and polarography [18]. Also, the determination of lovastatin and its hydroxy acid metabolite in plasma of either mouse or rat, by using liquid chromatography/ion spray tandem mass spectrometry has been described [21].

In our knowledge only a few studies concerning with its stability have been previously reported. Lovastatin at solid state and its oxidation products after exposure to an oxidative atmosphere has been determined by using capillary electrophoresis and liquid chromatography [22]. Also, the oxidation of lovastatin in the solid state and its stabilization with natural antioxidants [23] and the kinetic of its oxidation together with other anticholesteremic agents assessed by oxygen polarography have been described [24].

Taking into account these previous antecedents, in the present work an HPLC method was developed and applied to study the hydrolytic behaviour of lovastatin in different gastrointestinal dissolution media and simulated fluids, selected from United States Pharmacopoeia [25].

## EXPERIMENTAL

*Reagents and drugs.* Lovastatin (100% chromatographically pure) were obtained from Mintlab Laboratory (Santiago-Chile). The solvents employed were acetonitrile HPLC grade (99.8%, EMD Chemicals Inc.), methanol HPLC grade (99.9%, EMD Chemicals Inc.), ethanol p.a (99.8%, Merck), hydrochloric acid p.a (32%, Merck) and phosphoric acid p.a. (85%, Fluka). All other reagents employed were of analytical grade. The water was double distilled and deionised (Milli-Q quality).

*Buffer solutions.* Solutions for the HPLC studies were buffered using a 0.05-M buffer phosphate solution adjusted at pH 4 with phosphoric acid. For the degradation trials buffer solutions were prepared according to United States Pharmacopoeia [25].

*High Performance Liquid Chromatography (HPLC).* HPLC measurements were carried out by using a Waters assembly equipped with a 600 model Controller pump and a 996 model Photodiode Array Detector. The acquisition and data treatment were made by means of the Millenium version 2.1 software. As chromatographic column a μBondapak/Porasil C-18 column, 10-μm-particle size (3.9 mm×150 mm) and as column guards a C18 μBondapak (30 mm×4.6 mm) were employed. The injector was a 20 μL Rheodyne valve, model 7125.

*Chromatographic conditions.* An isocratic elution system composed by acetonitrile:methanol:phosphate buffer solution pH 4 (32%:33%:35%) at 45°C temperature, flow 1.5 mL/min and at a wavelength detection of 238 nm.

*Validation of the method.* Once chromatographic conditions were established, method validation was performed following the procedure described in General Chapter <1225> *Validation of Compendial Methods*, of the United States Pharmacopoeia [25], and hence the data elements required for validation are linearity, precision, accuracy and specificity.

*Linearity and range.* A stock solution of the drug ($1\times10^{-3}$ M) was prepared. This stock solution was diluted to prepare solutions containing $1\times10^{-4}$ M - $1\times10^{-6}$ M of the drug and injected in triplicate into the HPLC column.

*Precision.* Ten injections, of $5\times10^{-5}$ M lovastatin concentration were given on the same day and the values of relative standard deviation were calculated to determine intra-day precision (repeatability assay). These studies were also repeated on different days to determine inter-day precision (reproducibility assay).

*Accuracy.* Accuracy was evaluated by fortifying a mixture of decomposed reaction solutions with $5\times10^{-5}$ M lovastatin concentration. The recovery of added drug was determined.

*Specificity and selectivity.* The specificity of the method towards the drug was established through study of resolution factor of the drug peak from the nearest resolving peak. Overall selectivity was established through determination of purity for each degradation product peak using PDA detector.

Also, the detection (LOD) and quantitation limits (LOQ) of the method were calculated by using the average (*Yb*) and standard deviation (*Sb*) of the blank estimated response, calibration curve slopes (*m*) and with a signal/noise ratio of 3 and 10, respectively, according to the following expressions [26]:

$$LOD = \left[\frac{(Yb + 3 \cdot Sb)}{m}\right] \quad \text{and} \quad LOQ = \left[\frac{(Yb + 10 \cdot Sb)}{m}\right]$$

*Degradation trials.* Buffer solutions were spiked with lovastatin to obtain an initial concentration ranging between $1.0\times10^{-3}$ M and $5.0\times10^{-5}$ M. Solutions were divided in a number of 2 mL amber vials (one for each point of the degradation curve) and then placed in a oven at 80.0, 60.0 and 37.0°C (± 0.2 °C). Vials were taken from the oven at selected time intervals depending on both pH and temperature (i.e. each 5 min and 20 min for 0.1 N HCl and phosphate buffer pH 7.4 at 80°C, respectively). Immediately each sample was cooled on ice to quench the reaction and assayed by HPLC.

Degradation was monitored over at least three half-lives. Experiments were carried out in duplicate.

*Activation Energy (Ea).* Each Ea value was obtained from Arrehnius model, by plotting ln *k* vs. 1/T for each concentration tested. The final Ea value represents the average of the Ea calculated for 4 concentrations between $1\times10^{-3}$ M and $1\times10^{-5}$ M. In all cases regression coefficients have values higher than 0.997 were obtained.

## RESULTS AND DISCUSSION

Up to day, different analytical methods to determine lovastatin have been described. Such methods have been developed with the aim to be mainly applied to the determination of lovastatin in pharmaceutical dosage forms and in biological samples. In this work an HPLC method has been developed to be applied to assess the hydrolytic stability of lovastatin in different media.

*Development and optimisation of the stability-indicating method*

In Figure 2 typical HPLC chromatograms of standard lovastatin (Fig. 2A) and their degradation products after hydrolysis in different media (Figs. 2B-2C) are shown. Both C-8 and C-18 columns, different proportions of mixtures of acetonitrile/methanol/phosphate buffer solutions were tested as stationary and mobile phases, respectively. In the final selected conditions, lovastatin exhibited a retention time of 8.27±0.04 min. On the other hand, some differences in chromatograms corresponding to each degradation condition were obtained, thus lovastatin hydrolysis in alkaline media was instantaneous, with the appearance of a peak at Rt = 6.11±0.27 min and a shoulder near to 6.9 min. This characteristic does not make possible to follow its time-course under this condition (Fig. 2B). In acid media lovastatin generated two acid degradation products, with retention times of 5.94±0.78 min (2) and 3.54±1.86 min (1), (Fig. 2C). Furthermore, lovastatin degradation products retain the original UV-spectra of the parent drug, evidencing that the chromophore structure remains unaltered, which permits to conclude that only the ester and/or lactone moieties could be affected with the hydrolysis processes. These above results were obtained at the different temperatures which were as follows: 37, 60 and 80°C. As expected, parallel with the time-course of the hydrolysis, a decrease of the original peak corresponding to the parent drug occurred without any interference with other signals. Thus, the developed method becomes to be sufficiently selective to discriminate lovastatin from that corresponding to the hydrolytic products, representing a useful tool to follow this type of degradation.

*Validation of the developed stability-indicating method*

In Table 1, the analytical assessment of the new HPLC procedure for lovastatin is summarized. From the obtained analytical parameters it can be concluded that the developed chromatographic assay fulfils the analytical requirements, exhibiting an adequate repeatability and reproducibility (CV 0.057% and 0.73%, respectively) and a recovery higher than 98%. On the other hand, the concentration ranges for calibration curves seem to be adequate to follow degradation with detection and quantitation average limits of $9.1\times10^{-7}$ M and $2.8\times10^{-6}$ M, respectively.

*Degradation behaviour*

The developed method was successfully applied to determine the stability of lovastatin at different pHs, media and temperatures. The simulated gastrointestinal media and the others dissolution media were prepared as is described in the United States Pharmacopoeia [25].

*Order of reaction*

In Figure 3 the time-course of the lovastatin degradation in two different media is shown. As can be seen, the hydrolysis of lovastatin in acid medium follows a mixed kinetic. In contrast, in the other studied media the kinetic was of first-order. In the first case, the model to calculate the order of reaction only considered the experimental data point after 1 hr of degradation (Fig. 3A). In the other experimental conditions all the data points were used to fit the first-order kinetic as can be seen in Figure 3B.

To test the kinetic order of the hydrolytic degradation, experiments at both different initial concentration and pH were performed. As can be seen from Figure 4, changes in the initial concentration did not affect the slopes of the decay curves. Therefore, from the linearity of ln c *vs.* t plots ($r > 0.9991$) and

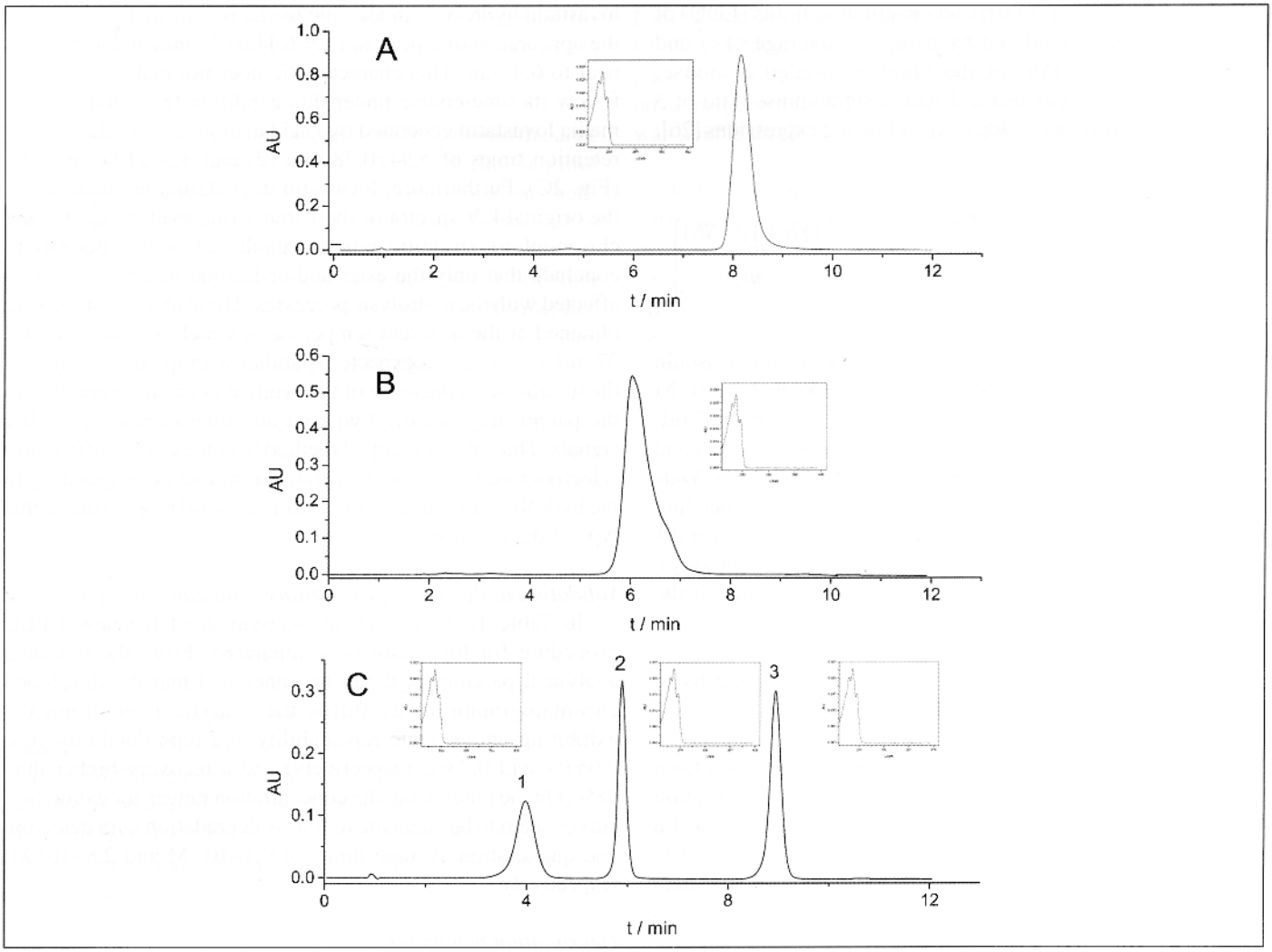


**Figure 2.** Chromatogram of (A) $3.0\times10^{-4}$ M lovastatin standard solution; (B) $3.0\times10^{-4}$ M lovastatin solution in 0.1 N NaOH; (C) $3.0\times10^{-4}$ M lovastatin solution after 12 h of acid hydrolysis (0.1 M HCl) at 80°C (1, 2= degradation products; 3=lovastatin). Insert: the UV-spectra of each peak. (Isocratic elution system composed by acetonitrile-methanol-phosphate buffer solution (pH 4; 50 mM) (32:33:35, v/v)).

**Table 1.** Analytical parameters of the new developed HPLC method

| | |
|---|---|
| Repeatability, CV (%)[a] | 0.057 |
| Reproducibility, CV (%)[a] | 0.73 |
| Recovery (%)[b] ± s.d. | 98.6 ± 0.9 |
| Concentration range (M) | $1.0\times10^{-6}$ - $1.0\times10^{-3}$ |
| Calibration curve | AUC = $1.91\times10^{10}$[c]+ 57457, (r= 0.9997, n= 10) |
| Detection limit (M) | $9.1\times10^{-7}$ |
| Quantitation limit (M) | $2.8\times10^{-6}$ |

[a]Concentration level of $5\times10^{-5}$ M
[b]Average on a concentration level of $5\times10^{-5}$ M

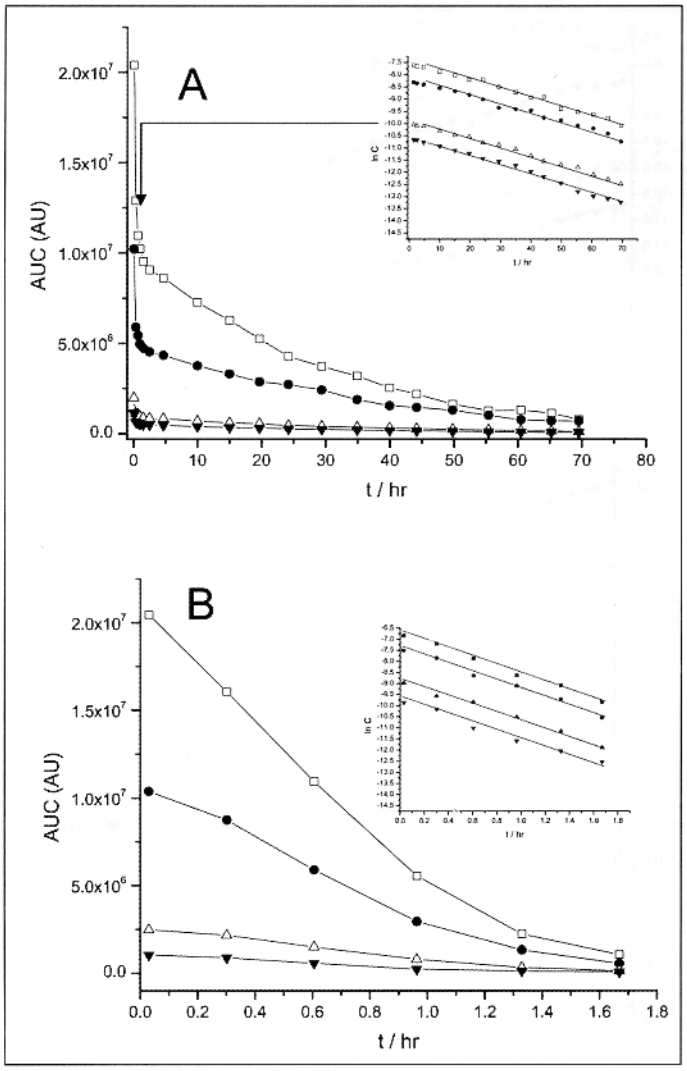


**Figure 3.** Time-course of lovastatin degradation at different initial concentration at 80°C in 0.1 M HCl (A), Phosphate buffer pH 7.4 (B). Insert: first order plots. $1\times10^{-3}$ M (□),$5\times10^{-4}$ M (●),$1\times10^{-4}$ M (Δ),$5\times10^{-5}$ M (▼) concentrations.

the fact that the slopes differences corresponding to ln c *vs.* t plots were not statistically significant ($p > 0.05$), as determined by bifactorial analysis, a pseudo-first order kinetic for the hydrolytic degradation for lovastatin can be assumed [27].

The time-course of products 1 and 2 is shown in Figure 5. As can be seen, the generation of product 1 is dependent of the media tested. Thus, in acid medium, this product is quickly generated during the first hour of hydrolysis. On the other hand, the product 2 is only observed in acid medium exhibiting poor chromatographic properties, probably due to at its high polarity compared with both parent drug and product 1. This behaviour of product 2 was observed in all temperatures and media.

In Table 2, the calculated pseudo first-order constants are summarized. Comparison of k values in different experimental concentrations at each temperature tested (37, 60 and 80°C) revealed that the differences were not statistically significant according to the Student *t*-test with $p > 0.05$. However, the comparison between *k* values in the different hydrolytic media and each temperature demonstrated that the differences between the values were statistically significant with a $p < 0.024$. On the other hand, *k* values enhanced parallel with increasing of temperature. As can be seen from Table 2, sodium laurylsulphate produces a decrease in the degradation constant in 3.5, 2.0 and 3.3 times at 37°, 60 and 80°C, respectively, as was compared with phosphate buffer media. Probably, this effect could be explained by a competition phenomenon between sodium laurylsulphate and lovastatin by the hydrolyzing agent.

Furthermore, from this Table it is possible to establish a rank order of lovastatin stability as follows: simulated gastric medium without pepsin > 0.06 M HCl > 0.1 M HCl > phosphate buffer pH 7 + sodium laurylsulphate > phosphate buffer pH 7.4. This order is in agreement with both half-live and $t_{90}$ calculated for the three tested temperatures (Table 3). As can be seen, half-live is dramatically affected by both temperature and medium

**Table 2.** Pseudo first order constant (*k*) for lovastatin in different media

| Medium[a] | Degradation constant ($k$, $h^{-1}$)* | | |
|---|---|---|---|
| | 37°C | 60°C | 80°C |
| 0.1 N HCl | $(3.9\times\pm 0.1)\times10^{-4}$ | $(3.2\pm0.2)\times10^{-3}$ | $(3.4\pm0.3)\times10^{-2}$ |
| 0.06 N HCl | $(7.8\pm0.6)\times10^{-5}$ | $(2.1\pm0.1)\times10^{-3}$ | $(2.31\pm0.4)\times10^{-2}$ |
| Simulated gastric medium[a,b] | $(1.59\pm0.03)\times10^{-4}$ | $(2.0\pm0.2)\times10^{-3}$ | $(1.9\pm0.2)\times10^{-2}$ |
| Phosphate buffer, pH 7.4[a] | $(9.4\pm0.5)\times10^{-2}$ | $(6.1\pm0.8)\times10^{-1}$ | $(21.0\pm0.03)\times10^{-1}$ |
| Phosphate buffer + SLS pH 7.0[a] | $(2.7\pm0.1)\times10^{-2}$ | $(2.8\pm0.2)\times10^{-1}$ | $(6.6\pm0.4)\times10^{-1}$ |

[a]prepared according to USP

[b]without pepsin

*$k$ represents the average value for 4 different concentrations, statistically supported ($p<0.05$)

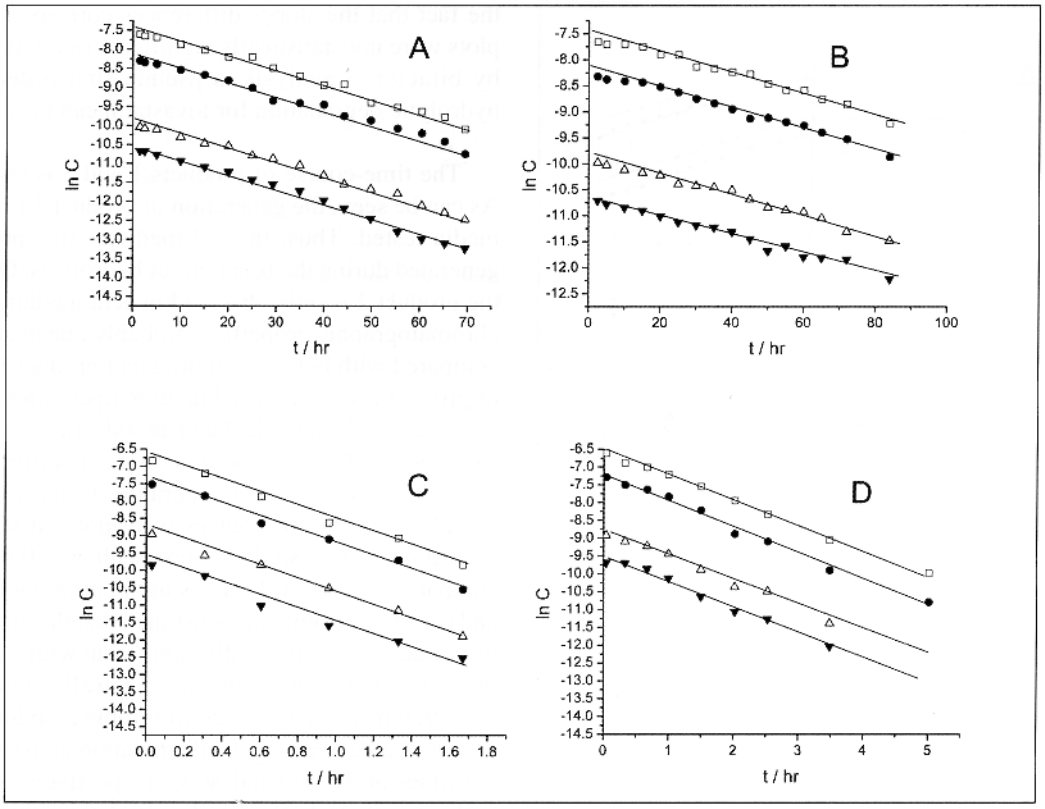


**Figure 4.** Decay plots of lovastatin hydrolysis at different initial concentration: $1\times10^{-3}$ M (□), $5\times10^{-4}$ M (●), $1\times10^{-4}$ M (Δ), $5\times10^{-5}$ M (▼), 80°C. A. 0.1 M HCl; B. simulated gastric medium; C. phosphate buffer pH 7.4; D. phosphate buffer + SLS pH 7.

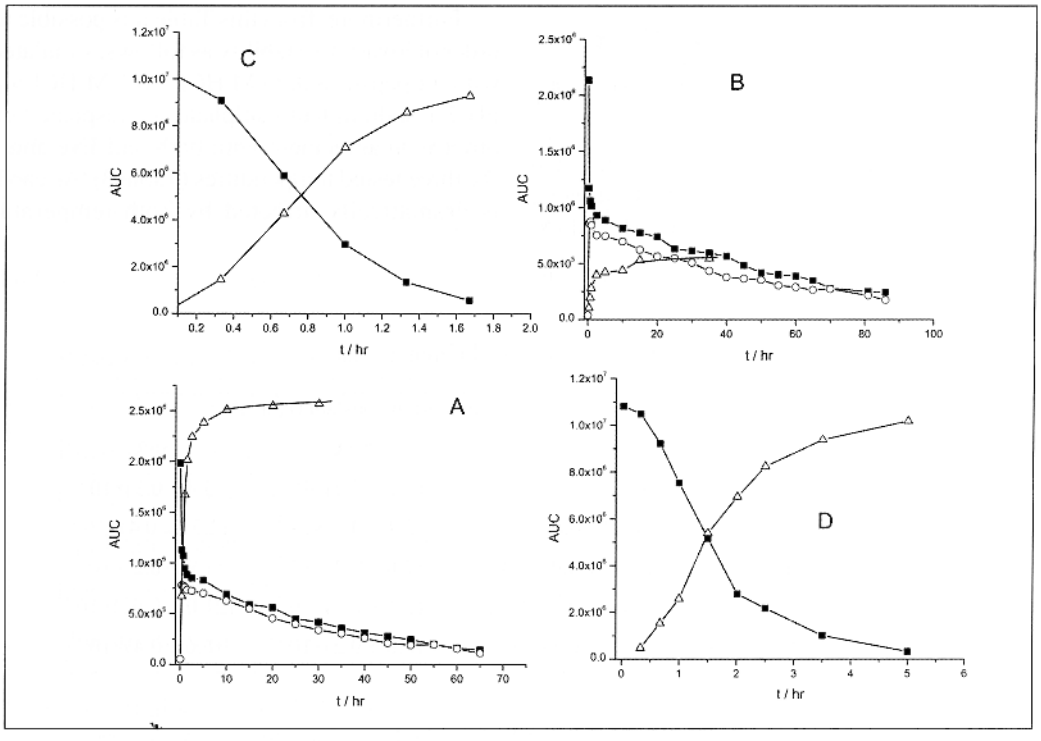


**Figure. 5.** $5\times10^{-4}$ M lovastatin hydrolysis at 80°C in 0.1 M HCl (A); simulated gastric medium without pepsin (B); phosphate buffer pH 7.4 (C) and phosphate buffer + SLS pH 7 (D). ■ Lovastatin; Δ product 1; □ product 2

**Table 3.** $t_{1/2}$ and $t_{90}$ values for lovastatin in different media*

| Medium[a] | 37°C | | 60°C | | 80°C | |
|---|---|---|---|---|---|---|
| | $t_{1/2}$ | $t_{90}$ | $t_{1/2}$ | $t_{90}$ | $t_{1/2}$ | $t_{90}$ |
| 0.1 N HCl | 18000 ± 50 | 272 ± 8 | 216 ± 10 | 33 ± 2 | 20 ± 2 | 3.1 ± 0.3 |
| 0.06 N HCl | $(90 \pm 7)\times10^2$ | $(14 \pm 1)\times10^2$ | 333 ± 10 | 51 ± 1 | 29.9 ± 0.5 | 4.5 ± 0.1 |
| Simulated gastric medium [a,b] | 4340 ± 69 | 658 ± 10 | 352 ± 31 | 53 ± 5 | 37 ± 3 | 5.6 ± 0.5 |
| Phosphate buffer, pH 7.4[a] | 7.4 ± 0.4 | 1.1 ± 0.1 | 1.2 ± 0.1 | $(1.9 \pm 0.1)\times10^{-1}$ | $(3.23 \pm 0.04)\times10^{-1}$ | $(4.88 \pm 0.05)\times10^{-2}$ |
| Phosphate buffer+ SLS pH 7.0[a] | 25 ± 1 | 3.8 ± 0.2 | 2.41 ± 0.05 | $(3.6 \pm 0.1)\times10^{-1}$ | 1.1 ± 0.1 | $(1.6 \pm 0.1)\times10^{-1}$ |

* $t_{1/2}$ and $t_{90}$ values are reported in hours.

[a]prepared according to USP

[b]without pepsin

**Table 4.** Activation energies (Ea) for lovastatin in different media

| Medium | $E_a$, Kcal/mol |
|---|---|
| 0.1 N HCl | 22.1±0.4 |
| 0.06 N HCl | 28.3±0.4 |
| Simulated gastric medium* | 23.8±0.2 |
| Phosphate buffer, pH 7.4 | 16.2±0.8 |
| Phosphate buffer + SLS pH 7.0 | 16.1±0.3 |

*Without pepsin

composition, i.e. lovastatin in phosphate buffer pH 7.4 at 80°C had only a half-live about 20 min. compared with that of the simulated gastric medium, in which exhibited a half-live about 37 hours. However, at a temperature of 37°C, the half-lives were about 7 hours and 6 months, respectively. Besides, typical pharmaceutical parameters that describe the shelf-life of a drug ($t_{90}$) were also calculated.

The Arrehnius activation energy (Ea) corresponding to lovastatin hydrolysis process in each media was calculated (Table 4). From these results (Tables 2 and 4) it can be concluded that the hydrolysis is faster in alkaline or neutral media than acid medium, which is consistent with the Ea values obtained for each media. On the other hand, the Ea values are in agreement with those reported for hydrolysis of ester groups [27]. Furthermore, taking into account the instantaneous hydrolysis in alkaline medium at room temperature, and both *k* and Ea values, seems to be that the hydrolysis process is base-catalyzed.

*Concluding remarks*

1. An HPLC stability-indicating method for lovastatin was developed.
2. Lovastastin exhibited a degradation pH-dependent with instantaneous hydrolysis in alkaline media at room temperature.
3. One or two degradation products could be observed when lovastatin is hydrolyzed in alkaline or acid medium, respectively.
4. Degradation products from lovastatin retain the UV-spectra of the parent drug, evidencing that the chromophore structure remains unaltered.
5. Lovastatin hydrolysis in different media follows a pseudo first-order kinetic.
6. The rank-order for lovastatin stability in different media is as follows: simulated gastric medium without pepsin > 0.06 M HCl > 0.1 M HCl > phosphate buffer pH 7.4 + sodium laurylsulphate > phosphate buffer pH 7.4.

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