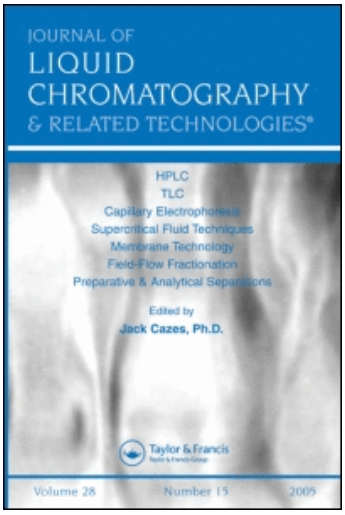

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FORCED DEGRADATION STUDIES OF SIMVASTATIN USING MICROEMULSION LIQUID CHROMATOGRAPHY

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□ *In this paper, the definition of simvastatin degradation profile by microemulsion liquid chromatography (MELC) is presented. The aim of the study was to investigate simvastatin stability after various stress tests, such as: acid and base hydrolysis, oxidation, and heat. The kinetics of acid and oxidative degradation was also studied. The complex retention mechanism occurring when microemulsion is used as eluent enabled the successful separation of a large number of degradants in prepared stress samples. The existence of a second partitioning site due to microstructural and interfacial characteristics of o/w microemulsion eluent facilitated HPLC separation. All the expected capabilities of MELC method were realized, demonstrating the advantage and value of the MELC method for a wide area of pharmaceutical analysis. For acid degradation the second order rate constant and half-life were calculated. Oxidative decomposition proved to be the first order reaction for which the rate constant and half-life were also determined.*

Keywords forced degradation studies, kinetic study, microemulsion liquid chromatography, simvastatin

INTRODUCTION

Forced degradation or stress testing is undertaken to demonstrate selectivity when developing stability indicating methods (SIM). These studies also provide information about the degradation pathways and degradation products that could form during storage. They may facilitate pharmaceutical development where the knowledge of a chemical behavior can be used to improve a drug product. It is important to be familiar with stereochemical stability, degradation product identification thresholds, polymorphism and crystal forms, stability of (parenteral) combination

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products, and mass balance, and that necessary information usually can be obtained with stress tests.[1]

The International Conference on Harmonization (ICH)[2] guidance provides very little information about strategies and principles for conducting forced degradation studies, including problems of poorly soluble drugs and exceptionally stable compounds. In particular, the issue of how much stress is adequate in stress testing is not addressed specifically. Overstressing a molecule can lead to degradation profiles that are not representative of real storage conditions and perhaps not relevant to method development.

Therefore, stress testing conditions should be realistic and not excessive. In this regard, it is the amount of stress that is important and not necessarily the extent of degradation. Indeed, some compounds may not degrade significantly after considerable exposure to stress conditions.

In this paper, forced degradation studies of simvastatin using microemulsion liquid chromatography (MELC) is described. Application of microemulsions as eluents in liquid chromatography represent an attractive alternative to conventional HPLC mode as this method enables the elution of very hydrophobic and hydrophilic (polar and ionic) analytes in the same chromatographic run without the need of gradient elution. Also, MELC proved to have a great potential due to its unique selectivity and high efficiency compared to conventional HPLC,[3–7] especially when structurally similar substances are analyzed. For the analysis of active substances and their degradation products in pharmaceutical preparations, MELC was also applied.[8–10] Due to the enhanced solubilisation capacity of microemulsions, MELC was successfully applied in bioanalysis, enabling direct injection of biological fluids after proper dilution with microemulsion eluent.[8,11]

All the advantages of the MELC method motivated the investigation of microemulsion eluent capability to separate the wide range of degradation products emerging from the stress studies. It can be expected that the degradation products arising from the stress studies would be either structurally similar or differ drastically in hydrophilic–lipophilic profile, as well as both. Some previous investigations conducted on simvastatin[5,12] inspired study in order to elucidate possible degradation pathways and make definite conclusions about simvastatin degradation behavior and profile. In the literature, stability indicating HPTLC and HPLC for simultaneous determination of ezetimibe and simvastatin were reported.[13,14] Also, the stability study of simvastatin under hydrolytic conditions assessed by liquid chromatography was done.[15] In presented SIM methods only partial forced degradation studies were done, e.g., in the reference[15] the authors gave only stability under hydrolytic conditions. As a very useful review of HPLC methods for the determination of simvastatin and

atorvastatin in bioanalytical assays, pharmaceutical assays and environmental applications can be found.[16]

A previously published paper[15] did not give complete insight in simvastatin degradation under various stress conditions. Therefore, the aim of this paper was to exploit all the capabilities of microemulsion eluent in order to examine the possible degradation pathways of simvastatin during forced degradation studies. Taking into account our previous investigation on simvastatin[12] with successful application of microemulsion eluent for simvastatin and its six impurities separation employing UV detection, the authors presumed the MELC method capability to separate substances of different polarity emerging during forced degradation studies. Also, for making definite conclusions about simvastatin degradation behavior and profile, the investigation was completed with the appropriate kinetic study.

EXPERIMENTAL

Chemicals

All reagents used were of an analytical grade. Sodium dodecyl sulphate (SDS), Tween 21 (polyoxyethylene sorbitan monolaurate) was obtained from Sigma (St. Louis, MO, USA). Diisoprophyl ether and *n*-butanol – HPLC grade were obtained from Riedel-deHäen (Sleeze, Germany). Water – HPLC grade, *di*-sodium hydrogen phosphate J. T. Baker (Deventer, Netherlands) and ortophosphoric acid Carlo Erba (Milan, Italy) were used to prepare an aqueous phase. The simvastatin and simvastatin-acid were of Ph. Eur. quality.

Chromatographic Conditions

The chromatographic system Waters Breeze consisted of Waters 1525 Binary HPLC Pump, Waters 2487 UV/VIS detector, and Breeze Software, Windows XP, for data collection. Separations were performed on the X-Terra^{TM} C_{18} 4.6 mm × 50 mm, 3.5 µm particle size column (Waters, USA) with UV detection at 238 nm. The flow rate was 0.3 mLmin^{-1}. The samples were introduced through a Rheodyne injector valve with a 20 µL sample loop. Mobile phase was prepared by mixing 0.9% *w*/*w* of diisopropylether, 1.7% *w*/*w* of sodium dodecyl-sulphate (SDS), 7.0% *w*/*w* of cosurfactant *n*-butanol and 90.4% *w*/*w* of aqueous 25 mM *di*-sodium phosphate pH 7.0 and treating them in an ultrasonic bath for 30 min.[12] The resulting transparent microemulsion was filtered through a 0.45 µm membrane filter Alltech (Loceren, Belgium).

Forced Degradation Studies

A stock solution of simvastatin with concentration of 1 mg mL^{-1} prepared in mobile phase was used in all degradation studies. Solutions for use in forced degradation studies were prepared by diluting the stock solution with sodium hydroxide or hydrochloric acid or hydrogen peroxide to achieve the final concentration of 100 μg mL^{-1} of simvastatin.

Kinetic Investigation of Acidic and Oxidative Degradation

A stock solution of simvastatin with concentration of 1 mg mL^{-1} was prepared in mobile phase. For kinetic studies solutions were prepared by diluting the stock solution with 0.01 M hydrochloric acid or 30% hydrogen peroxide to achieve the concentration of 100 μg mL^{-1} of simvastatin. The temperature was maintained at 25°C. The analysis of prepared solutions was carried out at regular time intervals.

RESULTS AND DISCUSSION

Forced degradation or a stress testing study is part of the development strategy and also an integral component of validating analytical methods that indicate stability and detect impurities.[2] The forced degradation studies are expected to elucidate possible degradation pathways, to identify the degradation products that may be spontaneously generated during drug storage and use, as well as to facilitate improvements in the manufacturing process and formulations in parallel with accelerated stability studies.

Stress studies may be useful in determining whether accidental exposure to conditions other than normal ranges (e.g., during transportation) are deleterious to the product. So they are normally carried out under more severe conditions than those used for accelerated studies.[2] The choice of stress conditions should be consistent with the product's decomposition under normal manufacturing, storage, and use conditions which are specific in each case.[17] Also choice of forced degradation conditions should be based on sound scientific understanding of the product's decomposition mechanism under typical use conditions, and usually to get a decomposition level of 10 to 15%, which is considered adequate for validation of a chromatographic purity assay. A minimal list of stress factors suggested for forced degradation studies must include acid or base hydrolysis, thermal degradation, photolysis, and oxidation.[18]

The analytical method that will be used in such analysis must be able to detect, separate, and must quantify all the observed degradation products,

although it is recognized that identification and characterization of the appropriate variants may require use of additional analytical methodologies. The MELC method demonstrated excellent capability for the separation of the mixtures composed of structurally similar substances as well as of very different polarity.[5] The complex retention mechanism occurring when microemulsion is used as eluent enables the successful separation of a large number of degradants that may occur in prepared stress samples. Existence of a second partitioning site due to microstructural and interfacial characteristics of *o/w* microemulsion eluent will facilitate HPLC separation.[6] In fact, all the analyzed substances will partition between mobile and stationary phase, such as the charged oil droplet and the aqueous buffer phase. Potentially formed degradants with hydrophobic properties will favor inclusion into the oil droplet rather than into the buffer phase, and as the surface of the droplet is not so rigid they can penetrate easily. Hydrophilic degradants that would probably form under oxidative stress will reside predominantly in the aqueous phase and their separation will be primarily governed by the stationary phase of the column packing material. Presence of the cosurfactant *n*-butanol influences the composition and thickness of interfacial film, affecting mobile phase hydrophobicity and changing the hydrophobic interaction between solute and stationary phase. The hydrophobicity of the microemulsion eluent has a large effect on the solubilization and partitioning of the investigated substance leading to changes in separation. The eluent in this study contains a large number of small droplets and is crucial to having a favorable influence on separation selectivity.[6]

All our previously performed investigations[5,6,12] induced the investigation of microemulsion eluent capability to separate the wide range of degradation products emerging from the stress studies conducted on simvastatin. In fact, it was confirmed that during simvastatin and its six impurities analysis by MELC in conjunction with UV detection, substances of different polarity and structural similarity would be eluted in 25 min. The most polar compound, simvastatin acid, was eluted first and the most lipophilic simvastatin dimer was eluted last. Moreover, in between these two extremes all the substances with structure similarity with simvastatin will elute. That means, the method could be successfully applied for the analysis of all possible degradation products of simvastatin emerging under various stress conditions. Also, MELC for simvastatin and its impurities analysis was fully validated and applied for analysis of pharmaceuticals.[12] The important data from method′s validation, necessary for current analysis, are given in Table 1.

It was expected that simvastatin as lacton will be very susceptible to hydrolysis (Fig. 1). So it was, and chromatographic analysis of a sample prepared in 0.01 M NaOH confirmed immediate and complete degradation of

TABLE 1 Part of Data from Method's Validation Important for Stress Studies [12]

	Validation Parameters	Simvastatin	Simvastatin-Acid	Anhydro Simvastatin
Linearity	$y = ax + b$	$130.083x + 1.483$	$0.137x + 0.0005$	$0.0722x - 0.0001$
	r	0.9990	0.9992	0.9985
	Sb	0.525	0.005	0.003
	tb	2.820	0.887	0.603
Intermediate Precision	Concentration	0.12, 0.16 and 0.20 mg mL^{-1}	1.2, 1.6 and 2.0 µg mL^{-1}	0.9, 1.2 and 1.5 µg mL^{-1}
	CV (%)	1.6% – 1.9%	1.6% – 3.0%	2.2% – 2.6%
	R (%)	100.0% – 102.5%	100.0% – 101.7%	100.0% – 101.7%
Reproductivity	Concentration	0.12, 0.16 and 0.20 mg mL^{-1}	1.2, 1.6 and 2.0 µg mL^{-1}	0.9, 1.2 and 1.5 µg mL^{-1}
	CV (%)	2.6%	1.9% – 2.6%	1.7% – 2.6%
	R (%)	96.3% – 96.7%	96.5% – 97.5%	93.3% – 96.7%
Limits	LOQ (ng mL^{-1})	10	10	30
	LOD (ng mL^{-1})	5	5	20

simvastatin to a corresponding acid at room temperature. Because of total hydrolytic degradation under these initial conditions, further increase of alkali strength and temperature would be totally useless and unnecessary.

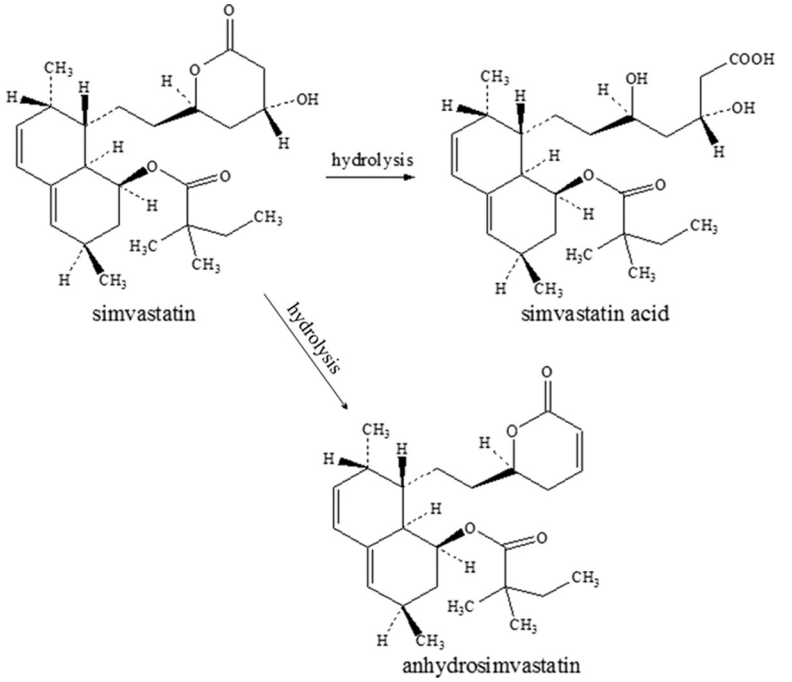

FIGURE 1 Possible degradation path-ways of simvastatin.

Acid stress with 0.01 M HCl led to 50% degradation after 30 min at room temperature and the main degradation product was simvastatin acid (Fig. 2b). The other possible degradation product that might appear during acid stress is anhydro simvastatin (Fig. 1). However, in the presence of 0.01 M HCl it was not produced, which was confirmed using appropriate standard substance.

Also, under this stress condition, mass balance was calculated. For calculation of the decrease of simvastatin concentration and increase of simvastatin acid concentration calibration curve parameters from Table 1 were used. It was confirmed that the only degradation product arising during the acidic stress was simvastatin acid. The calculated mass balance was 99.8% with the equal share of simvastatin and simvastatin acid.

An aqueous solution of simvastatin was heated at 70°C and after 40 min 30% of simvastatin was hydrolyzed to simvastatin acid (Fig. 2c). Confirmation of simvastatin acid's arise was done using the appropriate standard. Retention times of standard substance and substance which appear in the chromatograms, match in the precision of 0.05 min.

Oxidative stress was performed with 3%, 15%, and 30% H_2O_2 also. After 30 min 20% of simvastatin was degraded and according to retention times, a number of compounds more polar than simvastatin (Fig. 3) were eluted.

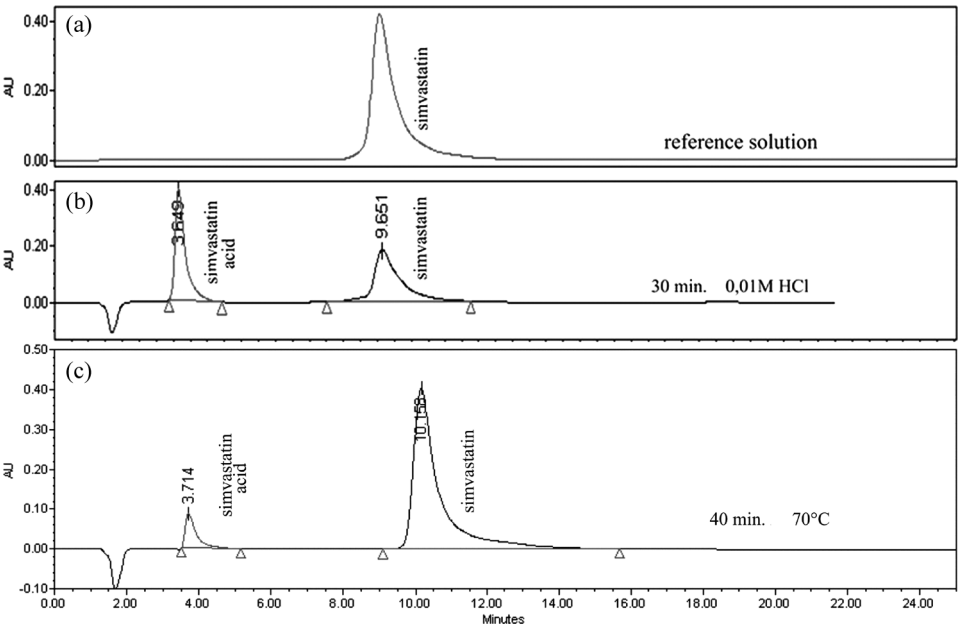

FIGURE 2 Chromatogram of simvastatin: (a) reference solution, (b) after acid stress and (c) after thermal stress.

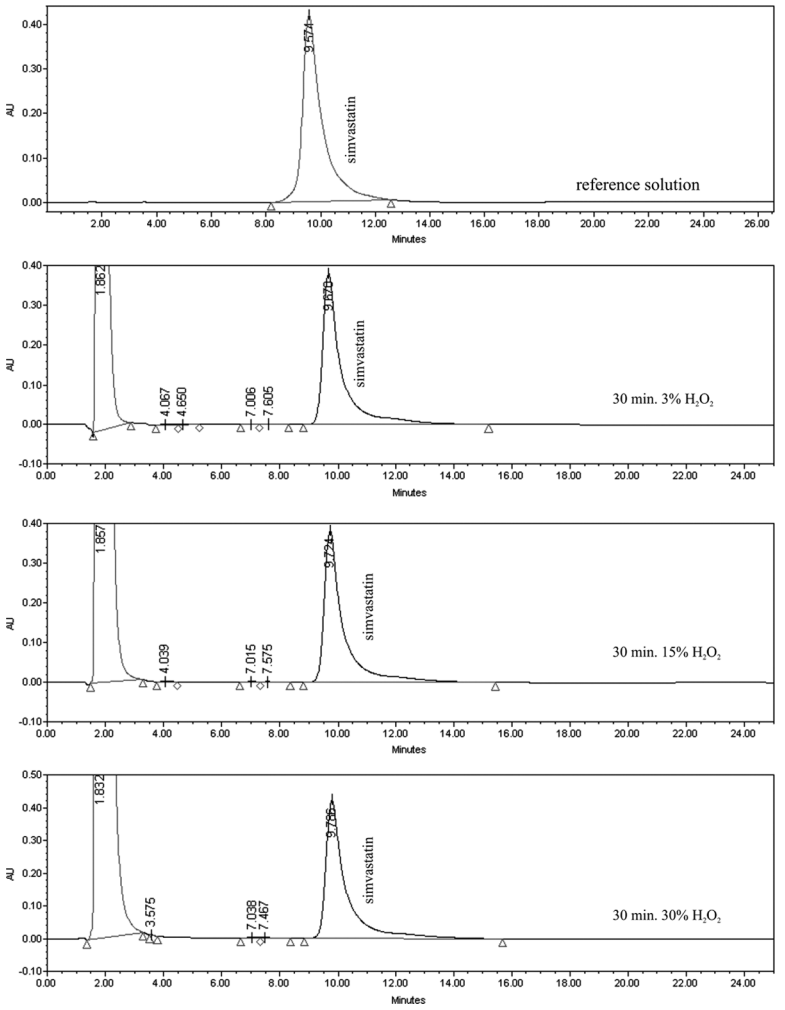

FIGURE 3 Chromatogram of simvastatin reference solution and after oxidative stress.

Increasing the concentration of hydrogen peroxide had no influence on the rate of oxidation.

Decrease of simvastatin was calculated employing the calibration curve from Table 1. Content of the others compounds which appeared was calculated with a normalisation procedure. Finally, in the case of oxidative stress, the calculated mass balance (% of remaining simvastatin + % of formed degradation products) was 98.5%.

In order to make definite conclusions about simvastatin degradation behavior and profile, kinetic studies were done. The primary aim of performing these studies for pharmaceutical compounds is to predict the rate of degradation reaction and to understand the mechanism of

the reaction.[19] Furthermore, understanding of these reactions provides valuable information as to which degradation products or by-products are likely to constitute significant impurities that need to be monitored. The majority of degradation reactions of pharmaceutical compounds in solution occurs at a finite rate and is affected by solvent type, concentration of reactants, temperature, pH of the medium, etc. The manner in which the reaction rate proceeds is dependent on the concentration of reactants and describes the order of the reaction.[19] The degradation of the majority of drugs can be classified as zero, first or pseudo first order, even though they may degrade by more complex mechanisms and the true expression may be of higher order.

The rate of reaction can be determined by measuring either the rate of decrease in the concentrations of the reactants or the rate of increase in the concentrations of the products. As a description of the reaction progress the rate law is used. There are two forms of a rate law for chemical kinetics: the differential rate law and the integrated rate law.[20] Each integrated rate law can be arranged as a linear equation:

$$[A] = -kt + [A]_0 \tag{1}$$

for zero order reaction, than:

$$\ln[A] = -kt + \ln[A]_0 \tag{2}$$

for first order reaction, and

$$\frac{1}{[A]} = kt + \frac{1}{[A]_0} \tag{3}$$

for second order reaction.

In the given equations $[A]$ represents concentration of the substance of interest at a particular time$t$, $[A]_0$ represents the initial concentration, and k is rate constant.

Arrangement of the integrated rate law in such manner can be used in the determination of reaction order and rate constant. The order can be determined by graphing each of the presented possibilities and determining which one is linear. In each of the three graphs, time is graphed on the x-axis while to test for a: 1) zero-order reaction, concentration of reactant is graphed on the y-axis; 2) first-order reaction, the natural log of reactant concentration is graphed on the y-axis and 3) second-order reaction, the reciprocal of reactant concentration is graphed on the y-axis. The appropriate one is only linear graph checked by the correlation coefficient (r). Once the correct linear graph is obtained and the reaction order is settled, the rate constant is determined from the slope of that line.

In this paper, kinetic studies for acidic and oxidative degradation were conducted as described in Experimental. On the basis of the experimental data the corresponding three kinetic plots were made and r was calculated to determine which is most linear. For acid degradation, the best fit line was obtained from the second-order rate equation indicating a second-order reaction (r was 0.6406, 0.9056, and 0.9968 for zero, first and second order rate, respectively). The value of the rate constant is the absolute value of the slope of second order line which is $1.78\,\mathrm{m}M^{-1}\mathrm{h}^{-1}$. The half-life for the second order acid degradation reaction (3.89 h) was calculated from the equation:

$$t_{1/2} = \frac{1}{k[A]_0} \tag{4}$$

Conversely, oxidative degradation proved to be first order as the best fit line was obtained by the first order rate equation (r was 0.8576, 0.9859, and 0.9447 for zero, first and second order rate, respectively). The value of the rate constant is the absolute value of the slope of the first order line which is $0.013\,\mathrm{min}^{-1}$. The half-life for the first order oxidative degradation reaction (53.3 min) is independent of the starting concentration and it is given by the equation:

$$t_{1/2} = \frac{\ln(2)}{k} \tag{5}$$

The variation in concentration with time provides a highly detailed description of how fast the reaction is occurring. Normally it is desirable to have a simple, approximate measure of the reaction rate, and the half-life provides such a measure. The faster the reaction, the shorter the half-life so it can be seen that the oxidative decomposition of simvastatin is faster than the acid degradation. Although the initial degradation in the acidic media is faster (50% degradation after 30 min at room temperature), after that the reaction slows down and all in all oxidative degradation proved to be faster. Nevertheless, conducted forced degradation studies confirmed the extreme instability of simvastatin. For that reason it should be preserved with the appropriate antioxidant in tight closed containers. As lacton, simvastatin is susceptible to hydrolytical degradation to the corresponding acid, so the contact with acids, base, or heat must be prevented.

CONCLUSION

The application of a MELC method in the forced degradation study of simvastatin was described. Microemulsion unique properties as separation

media enabled by the large number of nanometer sized spherical droplets, as well as the enhanced solubilization capacity make them perfect for stress studies. Microstructural and interfacial properties of microemulsion eluent facilitate the separation of a potentially large number of structurally different degradants originating from stress studies. The obtained results confirmed the extreme instability of simvastatin while the kinetic study conducted for acid and oxidative degradation indicate that these reactions are second and first order reactions, respectively. Approximate measure of the analyzed reaction was settled by the calculation of half-life for the acid (3.89 h) and oxidative degradation (53.3 min) and it was stipulated that the latter was a faster reaction.

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REFERENCES

1. Reynolds, D.W.; Facchine, K.L.; Mullaney, J.F.; Alsante, K.M.; Hatajik, T.D.; Motto, M.G. Available guidance and best practices for conducting forced degradation studies. Pharm. Technol. **2002**, *26*, 48–56.
2. ICH Stability testing of new drug substances and products. In: International conference on harmonization (2003) IFPMA, Geneva.
3. El-Sherbiny, D.T.M.; El-Ashry, S.M.; Mustafa, M.A.; Abd-El-Rahman El-Emam, A.; Hansen, S.H. Evaluation of the use of microemulsions as eluents in high-performance liquid chromatography. J. Sep. Sci. **2003**, *26*, 503–509.
4. Altria, K.D.; Marsh, A.; Clark, B.J. High performance liquid chromatographic analysis of pharmaceuticals using oil-in-water microemulsion eluent and monolithic column. Chromatographia. **2006**, *63* (7–8), 309–314.
5. Malenovic, A.; Ivanovic, D.; Medenica, M.; Jancic, B.; Markovic, S. Retention modelling in liquid chromatographic separation of simvastatin and six impurities using a microemulsion as eluent. J. Sep. Sci. **2004**, *27*, 1087–1092.
6. Malenović, A.; Ivanović, D.; Medenica, M.; Jančić, B.; Markovic, S. Influence of structural and interfacial properties of microemulsion eluent on chromatographic separation of simvastatin and its impurities. J. Chromatogr. A. **2006**, *1131*, 67–73.
7. McEvoy, E.; Donegan, S.; Power, J.; Altria, K. Optimisation and validation of a rapid and efficient microemulsion liquid chromatographic (MELC) method for the determination of paracetamol (acetaminophen) content in a suppository formulation. J. Pharm. Biomed. Anal. **2007**, *44*, 137–143.
8. Walash, M.I.; Belal, F.; El-Enany, N.; Abdelal, A. Microemulsion liquid chromatographic determination of nicardipine hydrochloride in pharmaceutical preparations and biological fluids. Application to stability studies. J. Liq. Chromatogr. & Rel. Technol. **2007**, *30* (8), 1015–1034.
9. El-Sherbiny, D.T.; Eid, M.I.; El-Wasseef, D.R.; Al-Ashan, R.M.; Belal, F. Analysis of flunarizine in the presence of some of its degradation products using micellar liquid chromatography (MLC) or microemulsion liquid chromatography (MELC) – Application to dosage forms. J. Sep. Sci. **2005**, *28* (2), 197–202.
10. Jančić, B.; Medenica, M.; Ivanović, D.; Malenović, A.; Marković, S. Microemulsion liquid chromatographic method for characterisation of fosinopril sodium and fosinoprilat separation with chemometrical support. Anal. Bioanal. Chem. **2005**, *383*, 687–694.

11. Jančić, B.; Ivanović, D.; Medenica, M.; Malenović, A.; Dimković, N. Development of liquid chromatographic method for fosinoprilat determination in human plasma using microemulsion as eluent. J. Chromatogr. A. **2005**, *1088*, 187–192.
12. Malenović, A.; Medenica, M.; Ivanović, D.; Jančić, B. Monitoring of simvastatin impurities by HPLC with microemulsion eluents. Chromatographia **2006**, *63*, S95–S100.
13. Dixit, R.P.; Barhate, C.R.; Nagarsenker, M.S. Stability-indicating HPTLC method for simultaneous determination of ezetimibe and simvastatin. Chromatographia **2008**, *67*, 101–107.
14. Chaudhari, B.G.; Patel, N.M.; Shah, P.B. Stability-indicating reversed-phase liquid chromatographic method for simultaneous determination of simvastatin and ezetimibe from their combination drug products. J. AOAC **2007**, *90*, 1242–1249.
15. Alvarez-Lueje, A.; Valenzuela, C.; Squella, J.A.; Nunez–Vergara, L.J. Stability study of simvastatin under hydrolytic conditions assessed by liquid chromatography. J. AOAC **2005**, *88*, 1631–1636.
16. Novakova, L.; Šatinsky, D.; Solich, P. HPLC methods for the determination of simvastatin and atorvastatin. Trends Anal. Chem. **2008**, *27*, 352–367.
17. Jenke, D.R. Chromatographic method validation: A review of current practices and procedures. II. Guidelines for primary validation parameters. J. Liq. Chromatogr. **1996**, *19*, 737–757.
18. Bakshi, M.; Singh, S. Development of validated stability-indicating assay methods – Critical review. J. Pharm. Biomed. Anal. **2002**, *28*, 1011–1040.
19. Ahuja, S. *Impurities Evaluation of Pharmaceuticals*; Marcel Dekker Inc.: New York, USA, 1998.
20. http://www.wwnorton.com/college/chemistry/gilbert/concepts/chapter14//ch14_3.htm#1.