Review Article

FORCED DEGRADATION STUDY OF STATINS: A REVIEW

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ABSTRACT

Forced degradation study is the degradation of new drug substances and drug products in more severe conditions than accelerated conditions. Forced degradation study were conducted to demonstrate the specificity of stability-indicating methods, providing insight into degradation pathways and drug degradation products, assisting in the elucidation of degradation product structures, identifying degradation products that could be spontaneously generated during storage and use of drugs and to facilitate improvement in manufacturing process and formulation corresponding with accelerated stability studies. Statins, a class of lipid-lowering medications, are the most widely prescribed drugs and an example of an unstable drug. Statins are susceptible to hydrolysis in the presence of high temperatures and humidity. Therefore, the review discusses various studies of forced degradation studies in six statins drug (atorvastatin, fluvastatin, pitavastatin, pravastatin, rosuvastatin, and simvastatin) to describe the drug's intrinsic stability thus it can assist the selection of formulations and packaging as well as proper storage conditions.

Keywords: Forced degradation study, Stress testing, Drugs stability, Statins

INTRODUCTION

The chemical stability of pharmaceutical molecules requires considerable attention because they affect the safety and efficacy of drug products [1]. The Food and Drug Administration (FDA) and International Council for Harmonisation (ICH) Guidelines state the requirements of stability testing data to understand how the quality of a drug substance and drug product changes over time under the influence of various environmental factors. Forced degradation is a process that involves the degradation of drug and drug products in more severe conditions than accelerated conditions and thus results in a degradation product that can be studied to determine molecular stability. In ICH guidelines, stress testing is intended to identify degradation outcomes that further assist in determining the intrinsic molecular stability and establishing degradation pathways, and to validate stability-indicating methods [2, 3].

Stress test should be consistent with product decomposition and specific manufacturing, storage, and normal use conditions in each case [4]. The choice of forced degradation conditions should also be based on a good scientific understanding of the mechanisms of decomposition of a product under typical usage conditions, and usually for a 10 to 15 % decomposition rate, which is considered adequate for the validation of chromatographic purity test [5]. The minimum stress factors suggested for forced degradation studies should include acid or base hydrolysis, thermal degradation, photolysis, and oxidation [6].

Statins, inhibitors of 3-hydroxy-3-methylglutarylcoenzyme A (HMG-CoA) reductase, is used to lower cholesterol by inhibiting the HMG-CoA reductase enzyme, which plays a central role in the production of cholesterol in the liver. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate, a rate-limiting step in cholesterol synthesis. Lovastatin, pravastatin, and simvastatin are inhibitors of HMG-CoA reductase derived from fungi, while atorvastatin, fluvastatin, pravastatin, pitavastatin, and rosuvastatin are completely synthetic compounds [7].

Statins have low bioavailability due to their poor aqueous solubility, low permeability, and high molecular weight of some of their members [8]. The bioavailability of statins varies from 5 % for simvastatin to 60 % or more for pitavastatin [9]. The drugs are divided into four classes in the biopharmaceutical classification system (BCS) based on its solubility. Solubility is a problem in Class II (i.e. simvastatin and atorvastatin) and IV drugs. There are several methods available to improve solubility, dissolution, and also bioavailability of drugs with poor solubility in water such as physical and chemical modifications, carrier system, micronization, etc. [10]. Formulation simvastatin nanoparticles with single emulsion diffusion method produce 50 % dose reduction without affecting its efficacy [11]. Atorvastatin has low bioavailability (12 %), so it is coated with sodium alginate as a hydrophilic polymer to increase its bioavailability [12]. Other research uses the principle of drug interactions with food/beverage. Oral administration of fresh liquorice drinks with atorvastatin, simvastatin, and lovastatin results in enhancement of drug bioavailability in healthy rats [13].

Statins are susceptible to high temperature and humidity because these drugs have a high risk of hydrolysis. For example, simvastatin is a common cholesterol-lowering drug known to be temperature sensitive. The simvastatin lactone ring is rapidly hydrolyzed into β-hydroxy acid, simvastatin acid, a process that is affected by the sample temperature [14, 15]. Therefore, this review discussed the study of forced degradation on six statins drugs (atorvastatin, fluvastatin, pitavastatin, pravastatin, rosuvastatin, and simvastatin). Based on the knowledge of the degradation process and its degradation results, it is expected to assist in the process of formulation, packaging selection, and determination of drug shelf life and storage conditions when the drug is distributed to the public.

Search criteria

Articles related to forced degradation study, stress testing, drug stability, and statin drugs were used in this review. Authors selected and took the important points from many references that published in 1996-2018.

Force degradation study of statin drugs

Force degradation study of atorvastatin

The degradation study of atorvastatin (ATV) was investigated by some researchers with a various method such as liquid chromatography-mass spectroscopy (LC-MS) [16], ultra performance liquid chromatography (UPLC) [17-19], and high-performance liquid chromatography (HPLC) [20-24]. Lakka *et al.* conducted stress tests on ATV with HPLC method. The stress tests were performed on the sample include reflux with 0.1 N HCl at 60 °C/30 min, reflux with 0.1 N NaOH at 60 °C/30 min, reflux with 1 % H_2O_2 at 60

°C/30 min, exposed to dry heat at 105 °C/6 h in the oven, reflux at 60 °C/30 min in water, exposed to visible light 1.2 million lux-hours, UV light 200 Watt-hours/m², and stored in humidity chamber at 25 °C/90 % RH for 7 d. Degradation was not observed in stress tests with visible light, UV, moisture, and hydrolysis of water. Acid hydrolysis, alkaline hydrolysis, and oxidative conditions showed no significant degradation. However, thermal stress indicated significant degradation [25]. Aiyalu *et al.* studied stress tests on ATV with high-performance thin layer chromatography (HPTLC) method. Stress tests were conducted in ATV sample such as neutral hydrolysis (80 °C, 1 h), acid (0.1 M HCl, 8 h, 80 °C), acid (1 M HCl, 8 h, elevated temperature (80 °C), base (0,1 M NaOH, 8 h, 80 °C), base (1 M NaOH, 8 h, elevated temperature (80 °C), oxidative (3 % H_2O_2, 6 h, room temperature), oxidative (30 % H_2O_2, 24 h, room temperature), dry heat (30 d, 50 °C), and photolysis (UV, 2 d). ATV had significant degradation in acid hydrolysis (86.14 % degraded at 80 °C, 89.63 % degraded at elevated temperature (80 °C)) and oxidative stress (52.23 % degraded at 3 % H_2O_2, 45.32 % degraded at 30 % H_2O_2) [26].

Goel *et al.* conducted a stress test with UPLC method. ATV was subjected to acid (1 N HCl, 25 °C, 5 min), base (0.01 N NaOH, 25 °C, 5 min), neutral (water, 80 °C, 4 h), strong oxidation (30 % H_2O_2, 80 °C, 1 h), thermal (80 °C, 1 d), and photolysis stress conditions (UV at 254 nm, 1 d). ATV was found to be sensitive to acid hydrolysis (80.9 % assay). The degradation study with 1 N HCl resulted in the lactone form of ATV as the primary degradation product in retention time (Rt) 1.941 min. ATV was stable in alkaline hydrolysis, in water at room temperature, in 30 % H_2O_2 at room temperature, in dry heat at 80 °C and UV light for one day. However, ATV was found unstable after water hydrolysis at 80 °C and produced a major degradation product in Rt 1.932 min. After heating in H_2O_2 at 80 °C, ATV produced two minor degradation products in Rt 2.292 and 2.661 min [27]. S. Naidu *et al.* performed the stress test on ATV with HPLC method. The samples were subjected to acid hydrolysis, alkaline hydrolysis, oxidation, and light stress. ATV was relatively stable at photolysis stress (UV, 24 h), slightly degraded on alkaline hydrolysis (0.1 M NaOH), oxidation stress (3 % H_2O_2), and acid hydrolysis (0.1 M HCl) [28].

Force degradation study of fluvastatin

Akabari *et al.* conducted a stress test on fluvastatin (FVS) with HPLC method. Samples for degradation studies underwent basic hydrolysis (0.1 M NaOH, 70 °C, 120 min), acid (0.1 M HCl, 70 °C, 120 min and 1 M HCl, 70 °C, 30 min), oxidative (3 % H_2O_2, 70 °C, 120 min), thermal (80 °C, 24 h), and photolysis (direct sunlight, 24 h). FVS was found susceptible to acid, alkaline hydrolysis, and oxidative stress. FVS was unstable and degraded rapidly (about 45 %/hour, 10.1 % assay) when exposed to acidic conditions in 0.1 M HCl. Base and oxidative stress resulted in 61.2 % and 43 % drug decomposition, a decrease in original peak area and no additional peaks were observed in the chromatogram. FVS underwent thermal and photolysis degradation slightly (2.65 % and 6.47 %) and no additional peaks were observed in chromatogram [29]. Gomes *et al.* conducted a degradation study on FVS by HPLC method. The sample had neutral hydrolysis (water, 80 °C, 2 h), chemical oxidation (3 % H_2O_2, 80 °C, 2 h), acid hydrolysis (1.0 mol/l HCl, 80 °C, 2 h), and alkaline hydrolysis (1.0 mol/l NaOH, 80 °C, 2 h). FVS was stable in neutral hydrolysis. However, after chemical oxidation test, there had been modified in the chromatogram. After acid and base hydrolysis, the chromatogram showed many additional peaks of degradation products [30].

Force degradation study of pitavastatin

Aglawe *et al.* studied a stress test on pitavastatin (PTV) with UV-Visible spectrophotometry method. The PTV stress test was performed in acid hydrolysis (0.1 N HCl, 5 h, 60 °C), basic hydrolysis (0.1 N NaOH, 5 h, 60 °C), neutral hydrolysis (water, 5 h, 60 °C), oxidative (3 % H_2O_2, dark conditions, 6 h), heat (60 °C, 12 h), and photolysis (direct sunlight, 12 h and UV light, 48 h). The degradation studies showed that PTV was degraded significantly under acidic, alkaline, neutral, photolysis, thermal, oxidation, and light stress conditions (23.35 %-82.31 % drug degraded) [31]. In another study, degradation study using HPTLC method was conducted. Samples

were exposed to acid (0.1 M HCl, 4 h, 75 °C), base hydrolysis (0.1 M NaOH, 2 h, 75 °C), oxidation (3 % H_2O_2, 2 h, 75 °C), heat (75 °C, 24 h), and photodegradation study (254 nm UV radiation, 24 h). PTV was sensitive to acid hydrolysis (degraded ±7.5 %/h) and an additional band (retention factor (Rf) 0.53) appeared in the chromatogram. PTV had stability under alkaline conditions and showed an additional band at Rf 0.55. PTV was stable in thermal, and UV (>90 % recovery). Chromatogram showed two additional bands (Rf 0.28 and 0.58) in oxidative stress and three additional bands (Rf 0.53, 0.58, and 0.61) in thermal degradation. Samples exposed to UV light showed an additional band at Rf 0.55 [32].

Damle *et al.* investigated PTV degradation results by HPTLC method. The drug was tested with stress on acidic (0.1 N HCl, 30 min, room temperature), base (0.1 N NaOH, 2 h, room temperature), neutral (water, 30 min, room temperature), oxidation (3 % H_2O_2, 2 h, room temperature), thermal degradation (80 °C, 6 h), and photolysis (UV light, 200 Watt-hours/m² and fluorescence light, 1.2 million lux-hours) conditions. PTV had significant degradation in photolysis conditions, thermal and alkaline hydrolysis. PTV underwent degradation slightly in acid hydrolysis, and the chromatogram showed one additional band at Rf 0.70. Mild degradation occurred in neutral hydrolysis and oxidative stress [33]. Panchal *et al.* performed a stress test with the HPLC method. The drug was tested with acid (0.01 M HCl, 1 h, 60 °C), base (0.01 M NaOH, 10 min, 60 °C), neutral (water, 1 h, 60 °C) hydrolysis, oxidation (0.3 % H_2O_2, 1 h, 60 °C), heat (60 °C, 4 h), and photodegradation studies (visible light, 12 h and UV light, 4 h). PTV was heavily degraded (47 %) in basic media and moderately degraded (27 %) in acidic media, yielding major and minor degradation products at Rt 2.60 and 3.90 min. Under neutral conditions, 10 % of the drug was degraded, with no major degradation products and two minor degradation products at Rt of 2.60 and 3.90 min. However, PTV was relatively stable in neutral conditions. PTV was 70 % degraded in oxidative stress, with no major degradation products and minor degradation products at Rt within 1.5-3.0 min. In thermal stress conditions, PTV was quite stable (11 % degradation) and minor degradation products were found at Rt range 1.5-3.0 min. After PTV was exposed to visible and UV light, the drug was degraded with 10 % and 9 % degradation, respectively [34]. A stress test by HPLC method was studied by Sujatha *et al.* The degradation studies were performed in acid hydrolysis (2 M HCl, 30 min, 60 °C), alkaline hydrolysis (2 M NaOH, 30 min, 60 °C), neutral hydrolysis (water, 6 h, 60 °C), oxidative (20 % H_2O_2, 30 min, 60 °C), thermal (105 °C, 6 h), and photolysis (UV room, 7 d). The degradation study obtained additional peaks in the chromatogram compared to standard PTV (Rt 3.823 min), acid hydrolysis (2 additional peaks at 2.889 and 5.143 min), alkaline hydrolysis (2 additional peaks at 2.733 and 13.376 min), neutral hydrolysis (an additional peak at 13.025 min), oxidative (2 additional peaks at 2.733 and 13.376 min), thermal (2 additional peaks at 5.366 and 6.071 min) and photolysis (2 additional peaks at 5.290 and 5.988 min) [35].

Force degradation study of pravastatin

Onal and Sagirli performed degradation study on pravastatin (PRV) with HPLC method. The stress conditions are carried out in acid hydrolysis (1 N HCl, 1 h, 80 °C), alkaline hydrolysis (1 N NaOH, 1 h, 80 °C), neutral hydrolysis (water, 1 h, 80 °C), oxidative (30 % H_2O_2, 1 h, 80 °C), thermal (105 °C, 5 h), and photolysis (366 nm UV light, 10 h). Neutral hydrolysis caused 10 % reduction in original drug peak and two additional peaks at Rt 1.53 and 3.20 min. In base hydrolysis, chromatogram showed ±90 % reduction of the original drug peak and two additional peaks at Rt 0.99 and 1.82 min. Under oxidative stress, the peak of the chromatogram was reduced±30 % of the original drug peak and a new peak appeared at Rt 1.54 min. In acid hydrolysis, the peak corresponding to the parent drug disappeared and two new peaks appeared at Rt 1.53 and 1.71 min. The chromatogram did not undergo any changes in thermal stress. Drugs degraded 50 % and new small peak appeared at Rt 1.60 min on UV exposure [36].

Athota *et al.* tested the degradation of PRV by HPLC method. Stress studies were performed in acid (0.1 N HCl, 30 min), base (0.1 N NaOH, 30 min), oxidative (30 % H_2O_2, 30 min), thermal (105 °C, 30 min), and photolysis (sunlight, 24 h). PRV was more degraded in acid hydrolysis

and less degraded in oxidative stress. The test results showed 4.35 % of the drug was degraded under acidic conditions, 4.21 % in basic, 2.20 % in oxidative stress, 3.27 % in thermal stress and 2.88 % in photolysis [37]. Ahmad *et al.* conducted a stress test by HPTLC method. Samples were tested with acid, base, oxidative, thermal, UV, and photolysis stress. Acid degradation was slower than the basic condition. The degradation products were observed at Rf 0.28 and 0.53 when the drug was heated with HCl 1 M at 80 °C for 8 h. The drug underwent very fast basic degradation in 1 M NaOH at 80 °C, about 80 % of the drug was degraded within 5 min and degradation products were observed at Rf 0.24, 0.40, and 0.52. Under oxidative stress (30 % H_2O_2, room temperature, 24 h), the degradation products were observed at Rf 0.24, 0.40, and 0.52. In UV radiation (254 nm), PRV was degraded slowly, yielding four additional peaks at Rf 0.29, 0.37, 0.55, and 0.78. The drug was almost stable in heat and sunlight, with additional peaks at Rf 0.53 and Rf 0.29 [38].

Force degradation study of rosuvastatin

The stress test on rosuvastatin (RSV) with a various method such as HPLC [39], UPLC [40], TLC [41], and UV spectrophotometry [42] was conducted in the previous study. Trivedi *et al.* conducted stress test with UPLC method in acid hydrolysis (0.1 N HCl, 80 °C, 2 h), alkaline hydrolysis (0.5 N NaOH, 80 °C, 6 h), oxidative (3 % H_2O_2, 80 °C, 6 h), thermal (100 °C, 8 h), and photolysis (UV light). No significant degradation was observed in oxidative stress, thermal stress, and alkaline hydrolysis. In contrast, significant degradation was observed in acid hydrolysis and UV light. Anti-rosuvastatin isomer and unknown impurities were formed [43]. Shah *et al.* conducted degradation study under stress conditions determined by ICH Guidelines with the HPLC method. Stress studies were performed in acid (0.1 N HCl), base (2 N NaOH), oxidative (30 % H_2O_2), thermal (50 °C for 21 d), and photolysis stress (fluorescence ~ 8,500 lux and UV light ~ 0.5 W/m²). Drugs degraded mainly in hydrolysis and photolysis conditions. Five degradation products were formed in acid hydrolysis and acidic photolysis conditions. One degradation product was formed in neutral and oxidative conditions. Two degradation products were produced at all photolysis conditions, including solid photolysis. The drug was stable against alkaline hydrolysis and thermal stress. The LC-MS analysis showed that five degradation products had the same molecular mass as in the drug, while the other six had a molecular mass of 18 Da less than the drug [44].

Ashfaq *et al.* studied RSV degradation with HPLC method. Stress conditions were performed under acid hydrolysis (5 M HCl, 60 °C, 4 h), alkaline hydrolysis (5 M NaOH, 60 °C, 4 h), oxidative (6 % H_2O_2, room temperature, 24 h), thermal (60 °C, 4 h), and photolysis (366 nm UV light, 10 h). RSV was highly degraded in acidic condition (40 %). At oxidative and basic stress, RSV had mild degradation with 6 % and 5 % degradation, respectively. Thermal stress had no effect on drug degradability [45]. However, other study stated that the drugs contained RSV and ezetimibe are highly sensitive towards alkaline conditions in comparison to other stress conditions [46]. Bellal *et al.* performed a stress test with the TLC method. The stress conditions were oxidative (10 % H_2O_2, 100 °C, 10 min), photolysis (UV light 254 nm, 30-120 min), thermal (100 °C, 10 min), acid (0.1 M HCl, 100 °C, 5 min), and base (0.1 M NaOH, 100 °C, 5 min) hydrolysis. Under acid hydrolysis, four major degradation products were detected with Rf values of 0.16, 0.27, 0.74, and 0.77. In alkaline hydrolysis, one major degradation product was detected at Rf 0.73. At thermal stress, two major degradation products were formed at Rf 0.74 and 0.77. The drug was more susceptible to oxidative stress than other conditions because five major degradation products are formed at Rf 0.15, 0.25, 0.30, 0.71, and 0.77. At photolysis stress, two major degradation products were detected at Rf 0.70, and 0.78 [47].

Force degradation study of simvastatin

The degradation in simvastatin (SIM) was conducted in previous study with HPLC method [48-51], UPLC (52) and UV derivative spectrophotometry [53]. Samples were tested with neutral hydrolysis (water, 100 °C, 4 h), acid (1 N HCl, 100 °C, 4 h) and base (1 N NaOH, 100 °C, 4 h), oxidation (30 % H_2O_2, 100 °C, 4 h), dry heat (105 °C, 4 h), and UV exposure (254 nm, 4 h). By HPLC method SIM was still observed in the presence of their degradation products after acid hydrolysis. However, SIM was more sensitive to alkaline hydrolysis and underwent complete degradation. The peak (Rt 4.2 min) after alkaline and neutral hydrolysis could be identified as hydroxyl acid form of SIM. After oxidation, the degradation product peak was detected at Rt 2.55 min and did not interfere with SIM retention time (Rt 6.5 min). At thermal stress and UV exposure, SIM did not show any degradation [54].

Ghodke undertook degradation study with HPLC method. The sample under various conditions such as neutral (water, 70 °C, 1 h), acid (3 M HCl, 70 °C, 6 h), basic (1 M NaOH, 80 °C, 3 h) hydrolysis, oxidation (30 % H_2O_2, 8 h, 25 °C), thermal (80 °C), and photolysis (exposure to short and long UV radiation, 48 h) conditions. Chromatograms of acid and base degradation showed an additional peak at Rt 6.2 min and Rt 6.3 min. Peaks of degradation product were not found in neutral hydrolysis, oxidative, thermal and photolysis stress conditions [55]. Chavhan and Ghante conducted the stress test on SIM with UV spectrophotometry method. The stability studies were carried out under acidic (0.1 N HCl, 60 °C, 3 h), base (0.1 to 5 N NaOH, 60 °C, 36 h), neutral (water, 60 °C, 3 h), oxidative (3 % H_2O_2, 60 °C, 14 h), thermal (80 °C, 4 h), and photolysis (sunlight, 4 h) conditions. SIM was found to be stable in basic (5 N NaOH up to 36 h) and photolysis conditions (in sunlight up to 8 d) when compared to other conditions. SIM was found to be unstable in acid, neutral, oxidative, and thermal conditions [56].

Sawant and Ghante reported SIM stability based on a study of forced degradation with HPLC method. The stability studies of the SIM were performed under acidic (0.1 N HCl, 80 °C, 3 h), base (0.1 N NaOH, 80 °C, 2 h), neutral (water, 80 °C, 3 h), oxidative (3 % H_2O_2, 60 °C, 14 h), thermal (50 °C, 21 d), and photolysis (fluorescence light 8500 lux-hours and UV light 0.5 Watt-hours/m² at 40 °C/75 % RH, 13 d) conditions. The drug was degraded under all stress conditions except photolysis. No significant degradation (<0.45 %) occurred in photolysis conditions. The degradation product of SIM was identified as 2 dihydroxy derivatives of SIM [57]. Malenovic *et al.* analyzed the degradation product using microemulsion liquid chromatography. The degradation was expected that simvastatin as lacton would be very susceptible to hydrolysis [58]. Nalaiya *et al.* tested the forced degradation of SIM with HPLC method. Active substances and finished products are tested with acid, alkaline, oxidation, heat, and photolysis stress conditions. Within 24 h, SIM was totally degraded under acidic conditions (1 N HCl, 50 °C) and 90 % degraded in its finished products. SIM was also degraded under oxidative stress (0.3 % H_2O_2, 50 °C) and neutral hydrolysis (water, 90 °C). However, SIM was relatively stable under alkaline hydrolysis (1 N NaOH, 50 °C) and photolysis (UV light, 24 h) [59].

CONCLUSION

Forced degradation studies provide knowledge of possible degradation pathways and degradation of active ingredients and help explain degradation structures. The degradation product resulting from forced degradation studies is a potential degradation product likely to be formed under suitable storage conditions. From the results of forced degradation studies on Statin drugs, all drugs have a tendency to degrade in all stressful conditions i.e. acid, base, neutral hydrolysis, oxidation, thermal and photolysis of a certain degree depending on the concentration and duration of stress exposure. This demonstrates the instability of statin drugs, so it requires special treatment starting from active ingredient formulation, packaging, distribution and storage of the final product.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICTS OF INTERESTS

All authors have none to declare

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