UTILIZING UPLC/MS FOR CONDUCTING FORCED DEGRADATION STUDIES

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INTRODUCTION

Chemical stability is one of the most important issues that impacts the quality and safety of a pharmaceutical product. The FDA and ICH require stability testing data to understand how the quality of an API or a drug product changes with time under the influence of environmental factors such as heat, light, and humidity.[1,2,4,6] Knowing the stability characteristics of a pharmaceutical allows for the establishment of storage conditions and shelf life, the selection of proper formulations and protective packaging, This is required for regulatory documentation. Forced degradation, or stress testing, is similar to stability testing but carried out under harsher conditions than those used for accelerated testing. Forced degradation is generally performed early in the drug development process and is the main tool used to predict stability related properties, to understand degradation products and pathways and to develop stability indicating methods.[3]

The most common analytical technique for monitoring forced degradation experiments is HPLC with UV and/or MS detection, allowing for peak purity, mass balance, and identification of degradation products. These methodologies are often time consuming and of medium resolution, requiring a significant analysis time to ensure that all of the degradation products are accurately detected. The use of UltraPerformance LC® (UPLC®)/UV/MS allows for faster and higher peak capacity separations which can aid in the analysis and identification of degradation products and shorten the time required to develop stability indicating methods. The purpose of this application is to demonstrate the advantages of resolution and sensitivity that UPLC brings to forced degradation studies.

EXPERIMENTAL

Forced degradation studies were carried out on Simvastatin under varied conditions of Acid/Base Hydrolysis, Thermal Degradation, Peroxide Oxidation, and Photo Degradation with the ultimate goal of achieving 10-20% degradation (loss of API). Additional degradation products to those normally observed in Real Time or Accelerated Stability Testing were generated. Acid and Base Hydrolysis and Peroxide Degradation were carried out on simvastatin in solution (10mM Ammonium Acetate, pH 4.5) while Thermal Degradation was determined on simvastatin solid. Photostability measurements were performed on both simvastatin solid and in solution. Solution degradation experiments were carried out at a simvastatin concentration of 1mg/ml. The degraded samples were diluted to a concentration of ~0.1mg/ml (1:10 dilution) prior to injection on the UPLC/UV/MS system. The data generated was used to monitor the effects of the stress conditions on the simvastatin.

LC conditions

LC System:	Waters® ACQUITY UPLC® System
LC Data Software:	Empower™ 2
Column:	ACQUITY UPLC BEH C18 Column 2.1 x 50 mm, 1.7 µm
Column Temp:	45 ˚C
Flow Rate:	600 µL/min.
Mobile Phase A:	10 mM Ammonium Acetate, pH 4.5
Mobile Phase B:	Acetonitrile
Gradient:	Linear Gradient 25-90% B over 7 min.

MS conditions

MS System:	ACQUITY™ SQD Mass Spectrometer
Ionization Mode:	ESI Positive
Capillary Voltage:	3200 V
Cone Voltage:	20 V
Desolvation Temp:	350 ˚C
Desolvation Gas:	900 L/Hr
Cone Gas:	50 L/Hr
Source Temp:	130 ˚C
Acquisition Range:	100—900 m/z (5000 Da/sec)

CONCLUSIONS

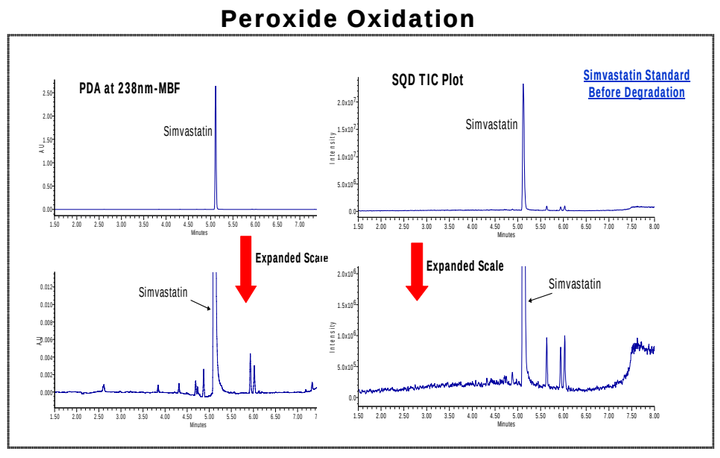
- **The ACQUITY UPLC®/PDA/SQD mass spectrometer was demonstrated to be an excellent tool for the separation of degradation products in a pharmaceutical product, such as simvastatin.**
- **The high peak capacity UPLC separations for complex mixtures of degradation products result in faster analyses, improve identification of impurity products and shorten the time required to develop stability indicating methods, improving the quality and throughput of forced degradation studies.**
- **The use of a multi-detection system is necessary to ensure that all degradation products formed are observed and identified.**

References

1. Guidance for Industry, Q1B, Photostability Testing of New Drug Substances and Products, Nov. 1996
2. Guidance for Industry, Q1A(R2), Stability Testing of New Drug Substances and Products, Nov. 2003
3. S. Upadrashta, 4th Annual Forced Degradation Conference, Las Vegas, NV
4. Y. Wu, Biomed. Chromatogr., 2000, **14**: 384-396
5. A. Alvarez-Luehe et al, J. of AOAC International, Volume 88, No. 6, 2005
6. H. Wang et al, J. Mass Spectrom., 2001, **36**: 58-70

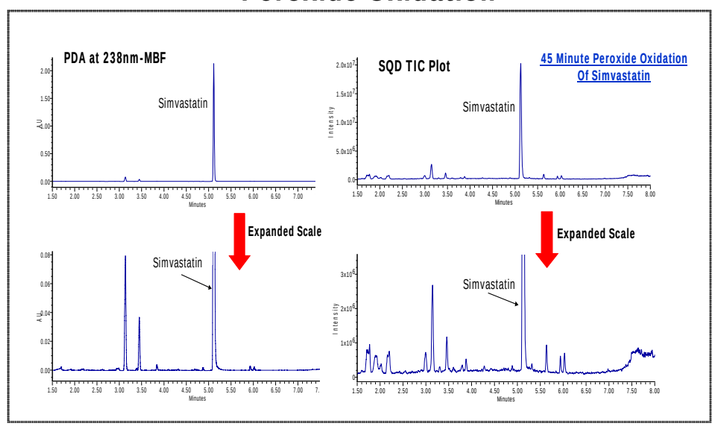
FORCED DEGRADATION METHODS

Peroxide Oxidation



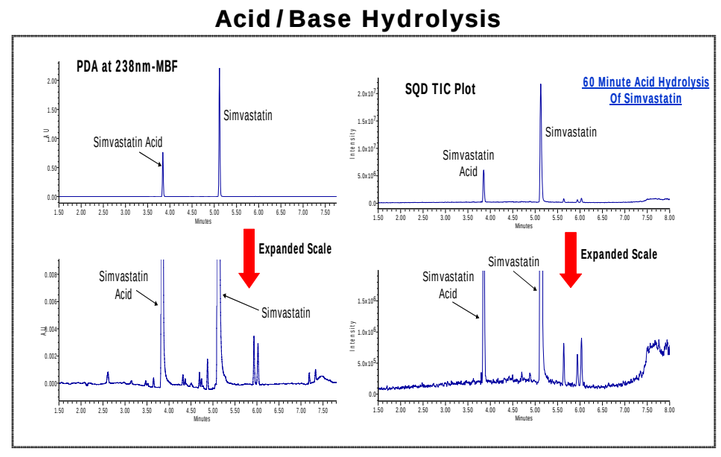
A standard solution of simvastatin was injected at a concentration of 0.1mg/ml as a control. The major peaks were identified and confirmed by oa-Tof MS to be the commonly observed impurities often found in simvastatin. This chromatographic data is the baseline that will be used to evaluate results of stress testing studies.

Peroxide Oxidation



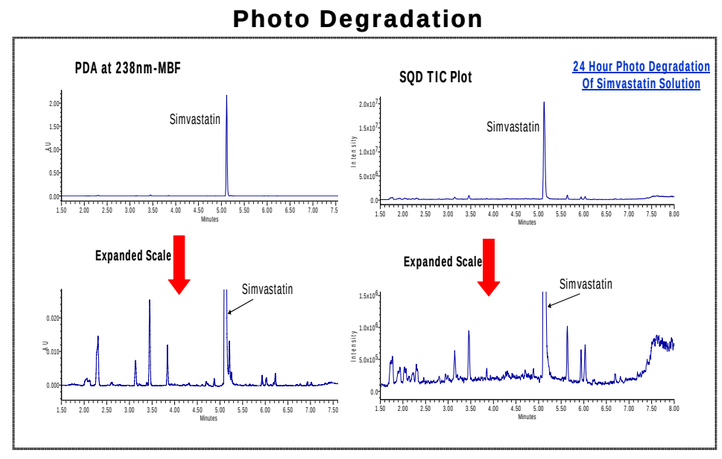
Oxidative degradation is most commonly achieved using peroxides, metal ions (metal salts), or radical initiators such as AIBN (autoxidation). This study found than a 7.5% Hydrogen Peroxide solution at 55°C for 45 minutes was sufficient to degrade the simvastatin by ~15% of intitial concentration resulting in many more degradation products than observed by simple acid or base hydrolysis.

Acid/Base Hydrolysis



The UPLC/UV/MS analysis of the acid and base hydrolysis of simvastatin showed it to be extremely sensitive to pH. Above pH 8, simvastatin rapidly undergoes hydrolysis to be completely converted to simvastatin acid, which agrees with previously published work.[5] This example demonstrates that hydrolysis of simvastatin in 100mM Hydrochloric acid for 1 hour results in the desired 10-20% loss of the initial API.

Photo Degradation



Photostability studies were performed on simvastatin in solution (1mg/ml in 10mM Ammonium Acetate, pH 4.5) with an exposure for 8 hours and 24 hours at the maximum Suntest CPS lamp intensity (583 and 1750 Watt-Hrs/m2, 320-400nm). In solution, the simvastatin exhibited significant degradation after 24 hours.

DEGRADATION RESULTS

Overlay of Degradation Product Profiles

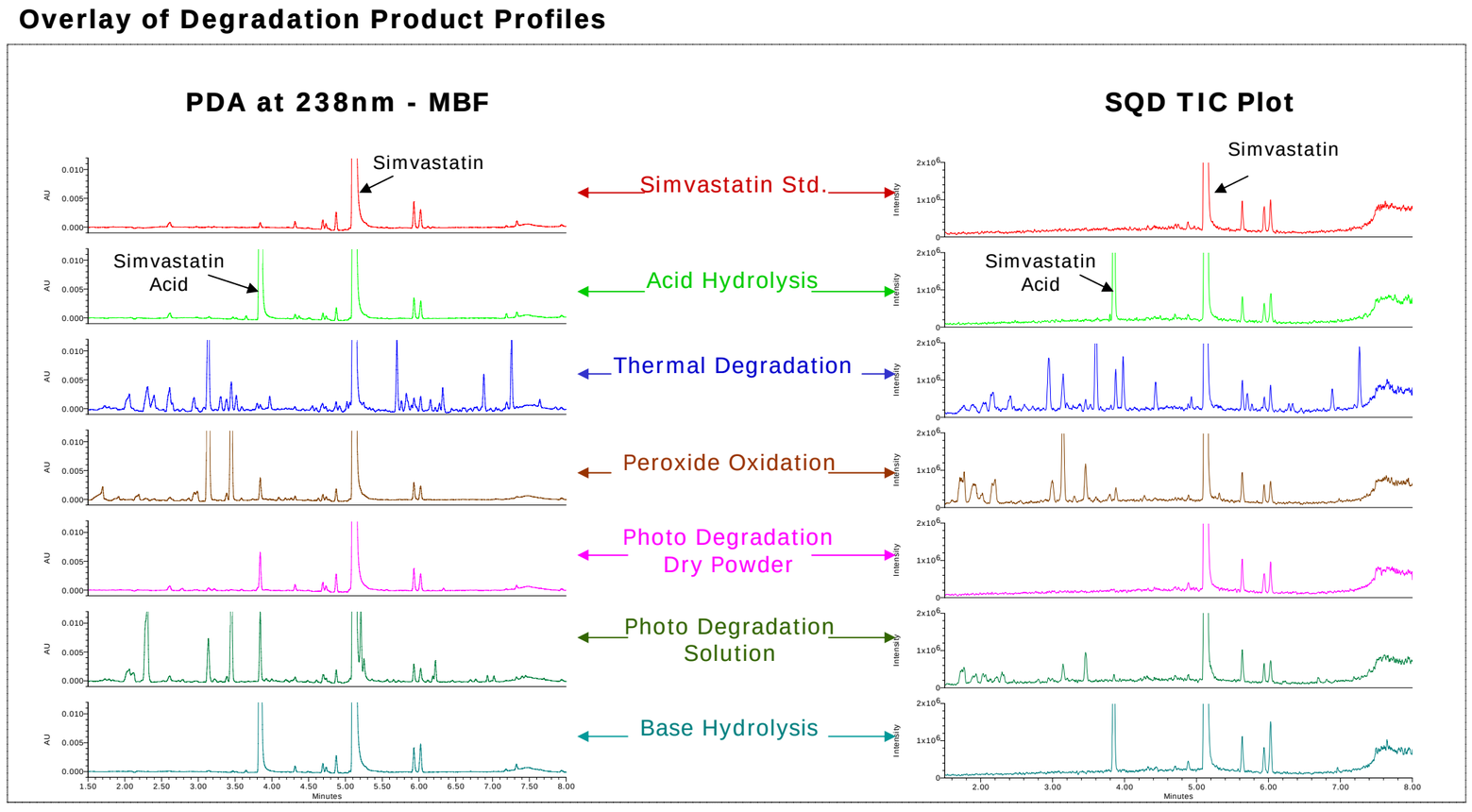


Table 1. Summary of Major Degradation Products Formed

Peak Rt (min)	Acid Hydrolysis		Base Hydrolysis		Temperature Degradation		Peroxide Oxidation		Photo Degradation	
	UV	MS	UV	MS	UV	MS	UV	MS	UV	MS
1.699							★			
1.760								★		★
1.886								★		
1.926										★
2.062					★				★	★
2.116									★	
2.161						★				
2.191								★		
2.308					★				★	★
2.397					★	★				
2.938						★				
2.986							★	★		
3.107					★			★		
3.137						★	★		★	★
3.449					★		★	★	★	★
3.594						★				
3.841	★	★	★	★			★	★	★	★
3.862						★				
3.970						★				
4.423						★				
5.209									★	
5.254									★	
5.699					★					
6.222									★	
6.322					★					
6.881					★	★				
7.257					★	★				

SUMMARY

The comparison of chromatograms obtained from analysis of the forced degradation of simvastatin by acid and base hydrolysis, thermal degradation, peroxide oxidation, and photo degradation (shown above) demonstrate the varied degradation product profiles that result from these various procedures. Although acid or base hydrolysis yield only simvastatin acid as a degradation product, other procedures such as photo and thermal degradation produce much more complicated and unique profiles of degradation products. The high efficiency separations obtained with ACQUITY UltraPerformance LC® systems allow for the easy and rapid analysis of these complex mixtures.

Figure 1 also demonstrates the utility of the combined detection of photodiode array and MS detection. Many of the degradation products were observed in the MS data channel only as the components do not contain chromophores thus are not detected by UV absorption. For other degradation products, UV detection was determined to be more sensitive than MS detection, this is mainly due to the lack of ionizable groups on the molecules. Table 1 lists the major degradation peaks observed for each degradation method including which type of detection was able to detect the degradation products. A multi-detector approach is clearly desirable in order to detect the maximum number of degradation products.

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