Design and Implementation of a Forced Degradation/Chemical Stress Study Using New LC Column Technology

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Introduction

A stability indicating method (SIM) is a quantitative analytical procedure used to detect a decrease in the amount of the active pharmaceutical ingredient (API) present due to degradation. A SIM is a validated analytical procedure that accurately and precisely measures active ingredients (drug substance or drug product) free from potential interferences like degradation products, process impurities, excipients, or other potential impurities, and the FDA recommends that all assay procedures for stability studies be stability indicating. It is recommended that forced degradation or chemical stress studies be carried out to determine if analytical methods are stability indicating prior to embarking on long term stability studies. SIM's are routinely developed by stressing the API under conditions exceeding those normally used for accelerated stability testing. In addition to demonstrating specificity in SIM's, chemical stress testing, also referred to as forced degradation, can also be used to provide information about degradation pathways and products that could form during storage, and help facilitate formulation development, manufacturing and packaging. Stressing the API in both solutions and in solid state form generates the sample that contains the products most likely to form under most realistic storage conditions, that is in turn used to develop the SIM. In simplest terms, the goal of the SIM is to baseline resolve all of the resulting products (the API and all the degradation products) each from the other (i.e. no co-elutions).

In this presentation, we discuss the experimental design and implementation of a protocol subjecting a simvastatin API to conditions typical of those used in studies of this type, including acidic, basic, thermal, hydrolysis and photostability (according to ICH guidelines) conditions. As a starting point, an existing USP HPLC method was evaluated. The existing method was then converted to newer, fused-core LC column technology and the two methods compared for resolution/efficiency and analysis time, and to determine if they are stability indicating.

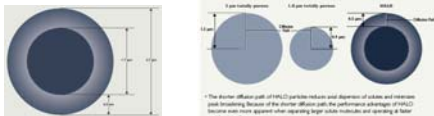


Figure 1: Halo® Fused-Core ® particle technology. Figures courtesy of Mac-Mod Analytical Inc.

Experimental

All work was performed on a Waters Alliance 2695 System, equipped with two detectors in series: a Model 2996 PDA and a Model 2487 variable wavelength UV monitoring 238 nm.

A 4.6 x 250-mm Xbridge C18 column (Waters) at a flow rate of 1.5 mL/min., and a 3.0 x 75-mm Halo column (Mac-Mod Analytical) at a flow rate of 0.6 mL/min. were used. Injection volumes of 40 and 5 µL were used on the Xbridge and Halo columns respectively. Both columns were operated at 45°C.

The mobile phase consisted of either 15 mM potassium phosphate or ammonium acetate (pH 4.5) and acetonitrile at the given proportions, mixed by the solvent manager.

Modern PDA technology is a powerful tool for evaluating specificity. PDA detectors can collect spectra across a range of wavelengths at each data point collected across a peak, and through software manipulations involving multi-dimensional vector algebra compare each of the spectra to determine peak purity. In this manner, PDA detectors today can distinguish minute spectral and chromatographic differences not readily observed by simple overlay comparisons. Figure 2 shows an example of a partial reversed phase LC separation, where, by all appearances, the peaks are certainly well resolved, sharp and symmetrical.

An examination (in Figure 3) of peak two indicated the peak was pure. However, a close examination of the spectral information related to peak one, reveals a different situation.

In Figure 4, the calculated peak purity (in green) is plotted against the noise threshold (in blue), both superimposed on the red chromatographic trace. The purity plot clearly indicates a co-elution in the front of the peak as the purity plot exceeds the threshold, and more method development work is necessary.

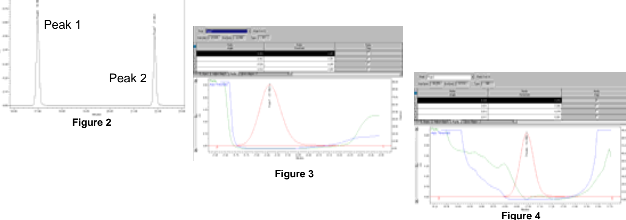


Figure 2

Figure 3

Figure 4

Figure 5: Comparison Chromatograms of Initial Forced Degradation Results on Halo Column

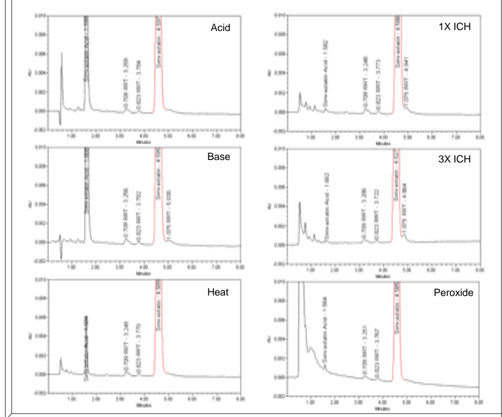
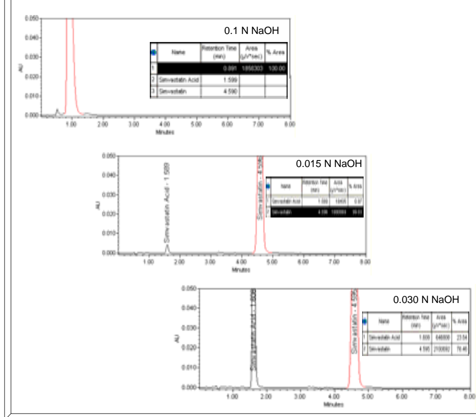


Figure 6: Base Study Comparison Chromatograms



Results and Discussion

The objectives of this work were two-fold: to determine if the USP method for simvastatin was stability indicating, and if a Halo column could be used to save time and increase throughput. In addition, an acetate buffer was substituted for the USP-specified phosphate buffer in anticipation of future MS work. A standard forced degradation/chemical stress study protocol was run and evaluated on both the USP-specified column and the Halo column. Figure 5 shows the chromatograms obtained on the Halo column using the acetate buffer for an acid and base-treated sample (without subsequent neutralization), and a heat and peroxide treated sample, as well as the photostability results. As seen, the major degradant is the acid form of simvastatin, with several other small impurities. The goal of a forced degradation study is to degrade the API by 5-15%; initially the base conditions resulted in complete degradation (Figure 6) and a more dilute solution of base had to be used to obtain the desired level of degradation. A direct comparison of the USP-specified column and the Halo column can be seen in Figure 7, for both the phosphate and acetate mobile phases using the 3x-photostability sample. This sample provided an impurity eluting in the tail of the API peak that was useful for column resolution and buffer comparisons. Using the original USP conditions, resolution for the small impurity peak was not as good on the Halo column; however switching to the acetate buffer and the corresponding decrease in organic strength yielded comparable results on the two columns. It is interesting to note that if the acetate buffer was applied directly at the USP-specified strength, the method would not be stability indicating due to the co-elution of the degradant in the tail of the API peak.

Conclusion

•An existing forced degradation protocol is a useful starting point, but has to be adapted according to the initial results obtained and the compound under study.

•In our hands, the PDA is a surprisingly useful tool in detecting potential co-elutions but is dependant on the strength and difference of the UV chromophore and how disparate the levels of the co-eluting compounds are.

•Converting to the Halo column and optimizing the conditions resulted in a stability indicating method with three times the throughput of the existing USP method, with comparable resolution and sensitivity.

Table 1: Example Table of Synomics Forced Degradation Study Conditions

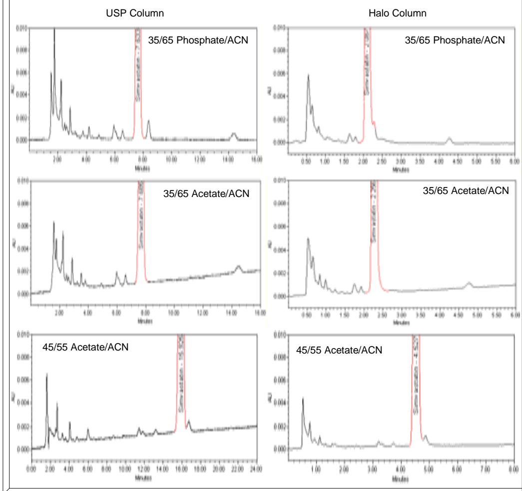
Procedure ID	Drug Substance	Reagent #1	Condition	Reagent #2	Diluent	Final Conc.
Drug Substance Control	0.5 mL of 5 mg/mL API Stock Solution	N/A	Immediately diluted to the final concentration	N/A	4.5 mL of Diluent per HPLC Method	0.5 mg/mL
Acidic Solution	0.5 mL of 5 mg/mL API Stock Solution	Add 1.0 mL 0.1N HCl	24 hrs. room temperature	Add 1.0 mL 0.1N NaOH	2.5 mL of Diluent per HPLC Method	0.5 mg/mL
Basic Solution	0.5 mL of 5 mg/mL API Stock Solution	Add 1.0 mL 0.1N NaOH	24 hrs. room temperature	Add 1.0 mL 0.1N HCL	2.5 mL of Diluent per HPLC Method	0.5 mg/mL
Acid/Base Control	NA	Add 1.0 mL 0.1N HCl	NA	Add 1.0 mL 0.1N NaOH	3.0 mL of Diluent per HPLC Method	0.5 mg/mL
Oxidative Solution	0.5 mL of 5 mg/mL API Stock Solution	Add 1.0 mL 3% H_2O_2	24 hrs. room temperature	N/A	3.5 mL of Diluent per HPLC Method	0.5 mg/mL
Oxidative Control	N/A	Add 1.0 mL of 3% H_2O_2	N/A	N/A	4 mL of Diluent per HPLC Method	0.5 mg/mL
Heat	0.5 mL of 5 mg/mL API Stock Solution	N/A	24 hrs at 60ºC	N/A	4.5 mL of Diluent per HPLC Method	0.5 mg/mL
Light #1	1.0 mL of 5 mg/mL API Stock Solution	NA	1X ICH Q1B	N/A	9 mL of Diluent per HPLC Method	0.5 mg/mL
Light #2	1.0 mL of 5 mg/mL API Stock Solution	NA	3X ICH Q1B	N/A	9 mL of Diluent per HPLC Method	0.5 mg/mL

Actual concentrations and diluent amounts depend upon experimental design and HPLC method. Goal is to have final concentration fully compatible with HPLC method.

Photostability

The photo-degradation samples were exposed in thin-walled, sealed quartz exposure vessels (50 mm diameter X 18 mm height) with silicone Teflon-lined caps utilizing the Atlas Suntest CPS+ (Model # 55007817) with a Xenon lamp and Q/Superax filter. This photostability chamber is designed to produce an output similar to the D65/ID65 emission standard (ICH light source Option 1). To minimize the effects of thermal degradation, an integral chiller unit cooled the inside of the exposure chamber. Protected samples (e.g., wrapped in aluminium foil) were used as controls and placed alongside the exposed samples to evaluate any potential contribution of thermally induced degradation to the total observed degradation. ICH Quinine Actinometry Standard Option 2 was used to determine the duration of the exposure needed to reach at least 1x and 3x ICH condition.

Figure 7: Photostability Results Comparison; Column and Buffer Changes



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