FORCED DEGRADATION STUDIES

Forced Degradation as an Integral Part of HPLC Stability-Indicating Method Development

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INTRODUCTION

High performance liquid chromatography (HPLC) is an integral analytical tool in assessing drug product stability. HPLC methods should be able to separate, detect, and quantify the various drug-related degradants that can form on storage or manufacturing, plus detect and quantify any drug-related impurities that may be introduced during synthesis. Forced degradation studies (chemical and physical stress testing) of new chemical entities and drug products are essential to help develop and demonstrate the specificity of such stability-indicating methods. In addition to demonstrating specificity, forced degradation studies can be used to determine the degradation pathways and degradation products of the APIs that could form during storage, and facilitate formulation development, manufacturing, and packaging. Procedures for the preparation of specific degradation products needed for method validation often emerge from these studies. For marketing applications, current FDA and ICH guidance recommends inclusion of the results, including chromatograms of stressed samples, demonstration of the stability-indicating nature of the analytical procedures, and the degradation pathways of the API in solid state, solution, and drug product. The chemical structures of significant degradation products and the associated procedures for their isolation and/or characterization are also expected to be included in the filing. The experimental protocol for performing forced degradation studies will depend on the active ingredients and formulation involved because the chemistry of each compound is different. In general, a target of approximately 10% degradation of the API during forced degradation, or exposure to energy in slight excess of what is typically used in accelerated storage is recommended. In this way, the "worst-case" degradation products can be studied. The following will provide some suggestions for performing forced degradation studies based upon available guidance from the ICH and FDA.

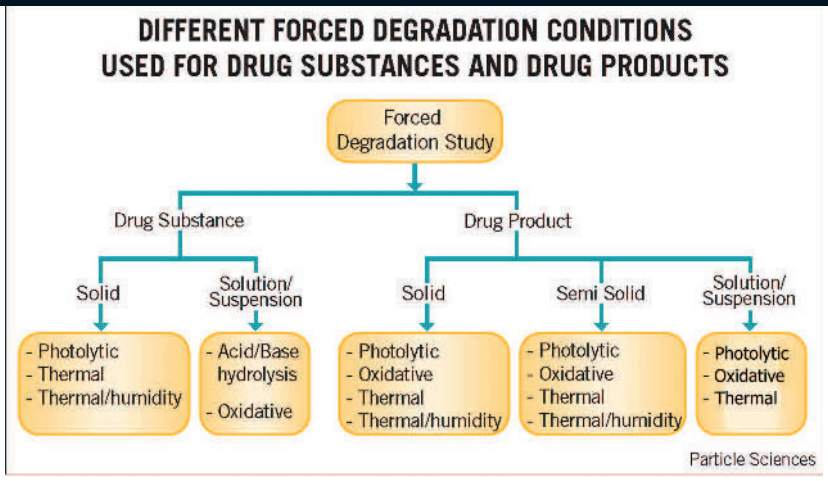
STABILITY-INDICATING METHOD (SIM)

According to an FDA guidance document, a stability-indicating method is "a validated quantitative analytical procedure that can detect the changes with time in the pertinent properties of the drug substance and drug product. A stability-indicating method accurately measures the active ingredients, without interference from degradation products, process impurities, excipients, or other potential impurities."[1]

Implicit in the aforementioned definition are the following: a SIM must be validated (demonstrate that it is suitable for its intended use), specific (resolution of active from related substances, peak purity), reproducible, quantitative, and able to monitor a change in the chemical, physical, and microbiological properties of drug product over time. The demonstration of specificity and the ability of the method to monitor a change in the chemical properties of the drug over time, invariably calls for a forced degradation (stress testing) study to be done on the drug substance and drug product.

Forced degradation on the drug substance and product will (in addition to establishing specificity) also provide the following

FIGURE 1



An illustrative flow diagram showing the different forced degradation conditions to be used for drug substances and drug products.

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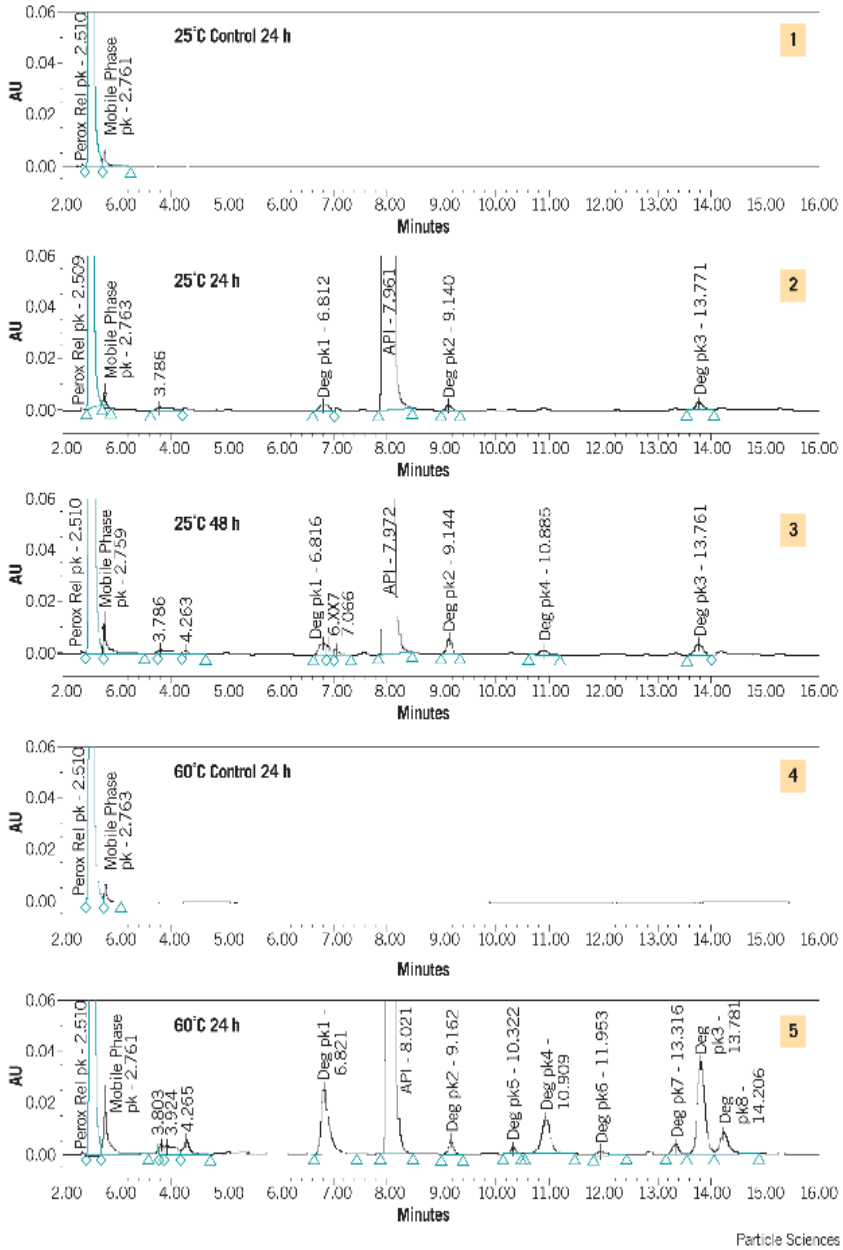
FIGURE 2

HPLC CHROMATOGRAPHIC SET

A sample HPLC chromatographic set showing the degradants generated on oxidative stress of an API using 3% peroxide at different temperatures and sampling times.



A sample chromatographic set showing the degradants generated for an API using 3% peroxide at different temperatures and sampling times.

information: (1) determination of degradation pathways of drug substances and drug products; (2) discernment of degradation products in formulations that are related to drug substances versus those that are related to non-drug substances (eg, excipients); (3) structure elucidation of degradation products; (4) determination of the intrinsic stability of a drug substance molecule in solution and solid state; and (5) reveal the thermolytic, hydrolytic, oxidative, and photolytic degradation mechanism of the drug substance and drug product.[2,3]

From the foregoing, it is obvious that forced degradation plays a key role not just in the development of stability-indicating methods, but also in providing useful information about the degradation pathways and degradation products that could form during storage. The information thus obtained will facilitate pharmaceutical development in areas such as formulation development, manufacturing, and packaging, where knowledge of chemical behavior can be used to improve the quality of drug product.

Despite the importance of forced degradation in pharmaceutical development, the current regulatory guidance documents governing forced degradation studies are very general.[1,2] One of the guidance documents, *Q1A (R2) – Stability Testing of New Drug Substances and Products*, states: "Stress testing is likely to be carried out on a single batch of the drug substance. The testing should include the effect of temperatures (in 10°C increments (ie, 50°C, 60°C) above that for accelerated testing), humidity (ie, 75% relative humidity or greater) where appropriate, oxidation, and photolysis on the drug substance. The testing should also evaluate the susceptibility of the drug substance to hydrolysis across a wide range of pH values when in solution or suspension."

This quotation demonstrates just how broad and unspecific these guidelines are. There are few practical instructions. For example, the guidance does not specify pH, temperature ranges, specific oxidizing agents, or conditions to use, the number of freeze-thaw cycles, and so on. Furthermore, the question of how much stress is adequate as well as when to begin stress testing is left up to the

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TABLE 1

CONDITIONS GENERALLY EMPLOYED FOR FORCED DEGRADATION

Degradation Type	Experimental Condition	Storage Condition	Sampling Time
Hydrolysis	Control API (no acid or base)	40 °C, 60 °C	1, 3, 5 days
	0.1N HCl	40 °C, 60 °C	1, 3, 5 days
	0.1N NaOH	40 °C, 60 °C	1, 3, 5 days
	Acid Control (no API)	40 °C, 60 °C	1, 3, 5 days
	Base Control (no API)	40 °C, 60 °C	1, 3, 5 days
	pH: 2, 4, 6, 8	40 °C, 60 °C	1, 3, 5 days
Oxidative	3% H_2O_2	25 °C, 40 °C	1, 3, 5 days
	Peroxide Control	25 °C, 40 °C	1, 3, 5 days
	Azobisisobutyronitrile (AIBN)	40 °C, 60 °C	1, 3, 5 days
	AIBN Control	40 °C, 60 °C	1, 3, 5 days
Photolytic	Light, 1 X ICH	NA	1, 3, 5 days
	Light, 3 X ICH	NA	1, 3, 5 days
	Light control	NA	1, 3, 5 days
Thermal	Heat Chamber	60 °C	1, 3, 5 days
	Heat Chamber	60 °C / 75% RH	1, 3, 5 days
	Heat Chamber	80 °C	1, 3, 5 days
	Heat Chamber	80 °C / 75% RH	1, 3, 5 days
	Heat Control	Room Temp.	1, 3, 5 days

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Conditions generally employed for forced degradation.

judgment of the pharmaceutical researcher. The following will provide some suggestions for performing forced degradation studies based upon available guidance from the ICH and FDA, thus narrowing these guidance generalities to practicalities.

APPROPRIATE TIMING

"If not performed earlier, stress studies should be conducted during Phase III to demonstrate the inherent stability of the drug substance, potential degradation pathways, and the capability and suitability of the proposed analytical procedures. The stress studies should assess the stability of the drug substance in different pH solutions, in the presence of oxygen and light, and at elevated temperatures and humidity levels. These one-time stress studies on a single batch are not considered part of the formal stability program. The results should be summarized and submitted in an annual report."[4]

The aforementioned quotation from the regulatory guidance document suggests that forced degradation studies could be delayed as late as Phase III clinical trials of the regulatory submission process. However, given the predictive nature of forced degradation studies, these studies are most beneficial if done initially in early development, ie, during the preclinical development or Phase I clinical trials. A forced degradation study on the drug substance at this stage will provide timely recommendations for improvements in the manufacturing process, ensure proper selection of stability-indicating analytical techniques, and ensure there is sufficient time for degradation product identification, degradation pathways elucidation, and optimization of stress conditions.[5] Such a proactive approach will help avert any surprises later in the development process.

HOW MUCH IS ENOUGH?

The question of how much stressing is enough has been the subject of much discussion amongst pharmaceutical scientists. In general, values anywhere between 5% to 20% degradation of the drug substance have been considered as reasonable and acceptable for validation of chromatographic assays.[6,7] However, for small pharmaceutical molecules for which acceptable stability limits of 90% of label claim is common, pharmaceutical scientists have agreed that approximately 10% degradation is optimal for use in analytical validation.[8] In the event that the experimental conditions generate little or no degradants due to the exceptional stability of the molecule, an evaluation should be made to verify if the drug substance has been exposed to energy in excess of the energy provided by accelerated storage (ie, 40°C for 6 months). If the answer is yes, then the experiment can be stopped and a note of the stability of the drug substance can be made. Unduly overstressing the drug substance may produce aberrant results.

EXPERIMENTAL DESIGN

In designing forced degradation studies, it must be remembered that more strenuous conditions than those used for accelerated studies (25°C/60% RH or 40°C/75% RH) should be used. At a minimum, the following conditions should be investigated: (1) acid and base hydrolysis, (2) hydrolysis at various pH, (3) thermal degradation, (4) photolysis, and (5) oxidation. For the drug substance and drug product, the scheme shown in Figure 1 could be used as a guide.[3]

The initial experiments should be focused on determining the conditions that degrade the drug by approximately 10%. The conditions generally employed for forced degradation are summarized in Table 1. However, some scientists have found it practical to begin at extreme conditions (80°C or even higher, 0.5N NaOH, 0.5N HCl, 3% H_2O_2)

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and testing at shorter (2, 5, 8, and 24 hrs, etc) multiple time points, thus allowing for a rough evaluation of rates of degradation.[9] Testing at early time points may permit distinction between primary degradants and their secondary degradation products. This strategy allows for better degradation pathway determination. It must be noted that a forced degradation study is a "living process" and should be done along the developmental time line as long as changes in the stability-indicating methods, manufacturing processes, or formulation changes are ongoing. Forced degradation is only considered complete after the manufacturing process is finalized, formulations established, and test procedures developed and qualified.

The conditions listed in Table 1 are by no means exhaustive and should be adjusted by the researcher as needed to generate ~10% degradation of the API. The nature (inherent stability/instability) of the particular drug substance will determine in which direction to adjust the stress conditions. Also, the aforementioned conditions could be used to stress the drug substance or drug product either in the solid or liquid/suspension form as applicable. The flow chart of Figure 1 should be followed as a guide.

As an example, sample chromatograms showing the degradants generated for an API using 3% peroxide at different temperatures and sampling times is shown in Figure 2. This was a scouting experiment to select the appropriate conditions for which a ~10% degradation will be generated. Chomatograms 2, 3, and 5 generated degradants totaling 5%, 11%, and 30% respectively. Therefore, the conditions for chromatogram 3 (3% peroxide at 25°C, for 48 hrs) were deemed suitable and were used for further method optimization.

For oxidative degradation with H_2O_2, at least one of the storage conditions should be at room temperature. Heating H_2O_2 solution increases the homolytic cleavage of the HO-OH bond to form the alkoxy radical (2HO●). The alkoxy radical is very reactive and may come to dominate the observed degradation pathway. Adding a small quantity of methanol in a confirmatory stress experiment quenches the alkoxy radical and rules out species produced by this more aggressive oxidizing agent. Also, the formation of peroxycarboxymidic acid has been observed when acetonitrile is used as a cosolvent in H_2O_2 stress studies (in basic conditions). The peroxycarboximidic acid has activated hydroxylation reactivity, which is not representative of H_2O_2. To circumvent these problems, some research scientists always perform a parallel or alternative oxidative study using azobisisobutyronitrile (AIBN), which is a less reactive oxidant and has been shown to produce more representative degradants.

SUMMARY

Forced degradation studies are indispensable in the development of stability-indicating and degradant-monitoring methods as part of a validation protocol. Forced degradation studies also provide invaluable insight in investigating degradation products and pathways of drug substances and products. Even though the ICH and FDA guidance documents only call for the inclusion of these studies in Phase III of the regulatory submission process, it is strongly recommended these studies be started as early as possible to be able to provide valuable information that can be used to assess the inherent stability of a drug, and to improve formulations and the manufacturing process.

Given that no specific set of conditions will be applicable to all drug substances and products, the pharmaceutical scientist should ensure the stress conditions are consistent with product decomposition under normal manufacturing, storage, and intended use conditions. Recommended stress factors include high and low pH, elevated temperature, photolysis, and oxidation. Care should be taken to avoid under-stressing or unduly over-stressing the drug substance or product, for this may lead to aberrant and non-representative results. A degradation level of approximately 10% of the drug substance should be optimal for method optimization.

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BIOGRAPHY

Dr. George Ngwa is an Analytical Chemist at Particle Sciences Inc. in Bethlehem, PA. At Orasure Technologies Inc. (Bethlehem PA), Dr. Ngwa's research focused on the development and validation of Chromatographic and Electrophoretic methods for the analysis and characterization of a wide range of small and large molecules. He earned his PhD in Pharmaceutical Chemistry from Lehigh University and has published and presented articles in national and international journals and conferences.