Available Guidance and Best Practices for Conducting Forced Degradation Studies

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This article summarizes the collective views of industry participants at a Pharmaceutical Research and Manufacturers of America Analytical Research and Development Steering Committee workshop on acceptable analytical practices on the topic of forced degradation studies. The article includes an overview of available guidance and some suggestions for best practices.

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Forced degradation or stress testing is undertaken to demonstrate specificity when developing stability-indicating methods, particularly when little information is available about potential degradation products. These studies also provide information about the degradation pathways and degradation products that could form during storage. Forced degradation studies may help facilitate pharmaceutical development as well in areas such as formulation development, manufacturing, and packaging, in which knowledge of chemical behavior can be used to improve a drug product.

The available regulatory guidance provides useful definitions and general comments about degradation studies. However, guidance concerning the scope, timing, and best practices for degradation studies is very general. Various issues related to stress testing are addressed in numerous guidance documents but not always in the context of stress testing. For example, the available guidance discusses issues such as stereochemical stability, degradation product identification thresholds, polymorphism and crystal forms, stability of (parenteral) combination products, and mass balance but does not address these issues in the context of degradation studies.

The FDA and International Conference on Harmonization (ICH) 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.

Also somewhat unclear is what should be done at each development phase from both a regulatory and a scientific perspective. Although FDA does not require degradation studies for an investigational new drug (IND) application, prelimi-

nary degradation studies are useful for the development of the stability-indicating methods that will be used during the clinical trials.

This article provides a practical interpretation and summary of the available guidance and some suggestions for best practices for conducting forced degradation studies. It represents the collective views presented by industry participants at the Pharmaceutical Research and Manufacturers of America Analytical Research and Development Steering Committee workshop about this subject. Concise summaries, rather than lengthy quotations, of what is stated in the guidance are provided with references. Material taken from the guidance is referenced and/or explicitly stated to be excerpted from the guidance.

Overview of regulatory guidance

According to the available guidance (1–3), forced degradation studies are carried out for the following reasons:

- development and validation of stability-indicating methodology
- determination of degradation pathways of drug substances and drug products
- discernment of degradation products in formulations that are related to drug substances versus those that are related to non–drug substances (e.g., excipients)
- structure elucidation of degradation products
- determination of the intrinsic stability of a drug substance molecule.

Degradation studies have several defining characteristics. They

- are carried out in solution and/or the solid state
- involve conditions more severe than accelerated testing (e.g., >40 °C; ⩾75% relative humidity; in excess of ICH light conditions; high and low pH, oxidation, etc.) (1,2)
- are typically carried out on one batch of material (1,2)
- include conditions that analyze thermolytic, hydrolytic, oxidative, and photolytic degradation mechanisms in the drug substance and drug product (as appropriate) (1,2)
- are not part of the formal stability program.

Summary of requirements at the IND phase

Although the reporting of degradation studies is not required in IND applications, preliminary studies may be carried out to facilitate the development of stability-indicating methodology. Studies can be conducted on the drug substance and developmental formulations to test for degradation by thermolysis, hydrolysis, oxidation, and photolysis or to evaluate the potential chemical behavior of the active ingredient.

A draft guidance document suggests that results of one-time stress studies should be included in Phase 3 applications for INDs (4).

Summary of requirements for marketing application

Completed studies of the degradation of the drug substance and drug product are required at the new drug application (NDA) stage, including isolation and/or characterization of significant degradation products and a full written account of the degradation studies performed (5).

Drug substance. For degradation studies of a drug substance, FDA requests the following at the time of registration:

- stressing the drug substance in solution or suspension at acidic and alkaline pH and under oxidation conditions (5)
- stressing the solid bulk drug substance at temperature and temperature–humidity conditions in excess of accelerated conditions (5)
- stressing the drug substance photolytically in the solid state and/or in solution in excess of ICH conditions (6)
- demonstration of the specificity of stability-indicating methods with forced-degraded samples or with the drug substance spiked with appropriate markers (7)
- isolation and/or full characterization (by means of NMR, mass spectrometry [MS], UV analysis, etc.) of all significant degradation products if possible (8). Procedures for the preparation and/or isolation (where applicable) and structure determination of the degradation products should be reported. Unsuccessful attempts to identify significant degradation products should also be documented (5).
- the chemical and physical properties of degradation products, if available (5)
- The mechanism and kinetics of formation of each degradation product, if available (5). The guidance says to determine reaction kinetics "if practicable," thereby acknowledging the difficulty in cases in which the mechanism may be complex (e.g., autoxidation).

Other issues that may be investigated but are not explicitly requested for degradation studies are the physical and chemical stability of important crystal forms, mass balance (1,2), and the formation of stereoisomers.

Drug product. The guidance specifies the following for degradation studies of the drug product at the time of registration:

- challenge methods intended for monitoring the stability of the drug product with the degraded samples (1) or with the drug substance spiked with a mixture of known degradation products
- isolation and characterization of significant (9) degradation products, if possible. The identity and chemical structure, procedure for isolation and purification, mechanism of formation (including order of reaction), and chemical and physical properties should be reported, if available (5). These degradation products include any drug substance–related compounds such as drug substance degradation products, drug substance–excipient degradation products, drug substance–extractive degradation products, and so forth.
- distinction between degradation products that are related to drug substances and those related to non–drug substances (9)
- photolysis of the drug product in excess of ICH light conditions (6).

Information requested in the submission. The available guidance (5) explicitly requests the following in the NDA documentation:

- for degradation products: identity and structure; procedure for isolation (where applicable) and characterization; mechanism of formation, including order of reaction; and physical and chemical properties
- information about stress testing of the drug substance and

Table I: General protocol for forced degradation studies (stress testing) of drug substances and drug products.

	Drug Substance		Drug Product	
Condition	Solid	Solution/ Suspension	Solid (Tablets, Capsules, Blends)	Solution (IV, Oral Suspension)
Acid/base		✓		X
Oxidative	X	✓	✓	✓
Photostability	✓	X	✓	✓
Thermal	✓		✓	✓
Thermal/humidity	✓		✓	

*✓ = recommended; X = optional, suggested for some compounds.

drug product (the guidance does not state specifically what information is required).

At the time of this writing (May 2001), more-specific guidance for the reporting of stress testing was found in FDA draft guidance documents dealing with stability (3) and method validation (10). According to these documents, the applicant should provide

- degradation pathways (3) of the drug substance, alone and in the drug product (10)
- a discussion of the possible formation of polymorphic and enantiomeric substances (10); the possible formation of any stereoisomers is implied
- data showing that neither the freshly prepared nor the degraded placebo interferes with the quantitation of the active ingredient (10)
- data from stress studies of the drug substance and drug product demonstrating the specificity of the assay and analytical procedures for degradation products (10). These data may take the form of representative instrument output (e.g., chromatograms) and/or degradation information obtained from stress studies (e.g., results of peak purity experiments performed on degraded samples).

Experimental approach

The experimental protocol for degradation studies of new drug substances and drug products ideally will result in samples that either have been degraded ~10% or have been exposed to an amount of energy that slightly exceeds that supplied under accelerated storage conditions (e.g., 40 °C for 6 months).

Forced degradation studies should be conducted whenever a stability-indicating method is required. Studies may need to be repeated as methods, processes, or formulations change. Alternatively, methods can be developed with a mixture of the known degradation products, if available (11). Forced degradation studies should be performed on each unique formulation before formal stability studies begin. Table I shows a general outline for degradation studies of new drug substances and drug products that was endorsed at the workshop.

Sufficient exposure of a drug substance or drug product is achieved when the drug substance has degraded ~10% from its initial amount or after an exposure in excess of the energy provided by accelerated storage (e.g., 40 °C for 6 months), whichever comes first. Application of this rule of thumb may result in no degradation in some cases. The goal is to generate a degradation profile that mimics what would be observed in formal stability studies under ICH conditions.

The duration of storage required at a given temperature can be estimated by making conservative kinetic assumptions. The energy of activation, E_a, represents the quantitative relationship between reaction rate and temperature. The range of activation energies for most drug substances is 12–24 kcal/mole with an average of about 19–20 kcal/mole (12). Assuming an activation energy of 12 kcal/mole affords nearly a doubling of the reaction rate for every 10 °C increase in temperature. (Recognize that this is a conservative estimate and that an activation energy of 20 kcal/mole affords about a 2.5- to 3-fold increase in reaction rate for every 10 °C increase in temperature.) With this relationship, one can calculate the amount of time a sample should be stored at a specified temperature to achieve the energy equivalent of exposure at accelerated stability conditions (e.g., 40 °C for 6 months). A sample of bulk drug substance stored at 80 °C would be stored for ~12 days (180 days ÷ 2^4). Thus, for a compound that degrades with E_a = 12 kcal/mole, storage at 80 °C for 12 days is kinetically equivalent to storage at 40 °C for 6 months.

Suggested equipment and exposure levels for photostability stress testing are described in ICH and FDA guidance (6). Light storage should be in sufficient excess of ICH light conditions.

The guidance states that solution-phase degradation studies of a drug substance can be carried out in solution or in suspension (5). The use of inert organic cosolvents may be indicated in cases in which drug substances are extremely insoluble but recognize the potential of any organic cosolvent to react with the drug substance under a given set of stress conditions.

For drug products, non–drug substance related peaks should be distinguished from drug substance related compounds (9). This process can be accomplished through comparative analysis of stressed samples of drug substance alone, of drug substance plus excipients, and of excipients alone.

Stressing drug substance and/or excipient blends instead of final dosage forms may be adequate for the determination of degradation pathways of a drug product. However, there may be significant differences in degradation profiles observed between blends and actual drug products. Consideration also should be given to the possibility of a reaction between the drug substance and components of a film-coating or capsule shell.

For combination parenteral or aerosol products, the guidance recommends an investigation of the chemical compatibility or stability of multiple actives that will be combined before administration (5). By extension, any combination product should be stressed to examine for drug–drug interactions potentially manifested by accelerated degradation and/or new degradation pathways and products. Early investigation can facilitate development of an optimum formulation as well as stability-indicating methodology.

Consideration may be given to stereochemical stability, mass balance, and crystal-form stability. Stereoisomers should be

treated like any other potential degradation product (13). Failure to observe an increase in stereoisomers during forced degradation studies may be sufficient justification to eliminate testing for stereoisomers during stability studies.

Investigation of the mass balance in degraded samples can reveal the adequacy of the analytical methods to examine for degradation products. Assessing mass balance is the process of adding the assay value and levels of degradation products to see how closely these add to 100% of the initial value, with due consideration of the margin of analytical error (1,2). In cases in which substantial mass loss is observed, efforts to account for the missing mass should be made such as consideration of response factors or the formation of highly retained or volatile degradation products. FDA recognizes that mass balance may not be obtained in all cases and instead emphasizes the thoroughness of the investigation to determine the specificity of the assay and the pathways of degradation (1).

The chemical and physical stability of crystal forms, if relevant, should be investigated. This analysis could include evaluation of stressed solid-state samples for changes in crystallinity or crystal form.

Method specificity for the active ingredient can be established by peak purity experiments using hyphenated techniques such as liquid chromatography (LC)–MS, LC–UV (photodiode array detection), and LC–NMR or orthogonal methods. If needed, degradation product structure elucidation can be accomplished with hyphenated techniques (e.g., LC–MS, LC–UV, LC–NMR) or by synthesis and/or isolation. Structure elucidation may be postponed until the drug demonstrates safety and efficacy.

A decision to isolate and/or characterize a degradation product should be based primarily on results obtained from formal stability studies of the drug substance and drug product whenever possible. The guidance provides the identification thresholds for degradation products in drug substances and drug products found in formal stability studies (8,9).

Summary

Forced degradation studies of new drug substances and drug products are essential to help develop and demonstrate specificity of stability-indicating methods and to determine the degradation pathways and degradation products of the active ingredients. They also can be useful in the investigation of the chemical and physical stability of crystal forms, the stereochemical stability of the drug substance alone and in the drug product and mass-balance issues, and for differentiating drug substance–related degradation products in formulations. Procedures for the preparation of specific degradation products needed for method validation often emerge from these studies. Knowledge gained from these studies can be used to guide formulation development and improve manufacturing and packaging processes.

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 drug substance in solution, solid state, and drug product. The structures of significant degradation products and the associated procedures for their isolation and/or characterization also are expected to be included in the filing.

The experimental protocol for degradation studies will depend on the active ingredients and formulation involved because the chemistry of each compound is different. A target of the lesser of 10% degradation of the active ingredient or exposure to energy in slight excess of accelerated storage is recommended. A compound may not necessarily degrade under a given stress condition. No further stressing is advised in these cases.

References

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2. FDA, "International Conference on Harmonization: Draft Revised Guidance on Q1A(R) Stability Testing of New Drug Substances and Products," *Federal Register* **65** (78), 21446–21453 (21 April 2000) [ICH Q1A(R)]. This revised guideline reached Step 4 of the ICH process on 8 November 2000. Implementation as of this writing — EU: adopted by CPMP, November 2000, issued as CPMP/ICH/2736/99. MHW: to be notified. FDA: to be notified.
3. FDA, "Draft Guidance for Industry, Stability Testing of Drug Substances and Drug Products," *Federal Register* (Notices) **63** (109), 31224–31225 (8 June 1998) (combination of ICH Q1A-Q1C and Q5C, draft).
4. FDA, "INDs for Phase II and III Studies of Drugs, Including Specified Therapeutic Biotechnology Derived Products," *Federal Register* (Notices) **64** (76), 19543–19544 (21 April 1999).
5. FDA, Center for Drug Evaluation and Research, "Submitting Documentation for the Stability of Human Drugs and Biologics" (Rockville, MD, February 1987).
6. FDA, "International Conference on Harmonization: Guideline for the Photostability Testing of New Drug Substances and New Drug Products," *Federal Register* **62** (95), 27115–27122 (16 May 1997) (ICH Q1B) and References 1 and 2.
7. It may be acceptable to use accelerated stability samples (e.g., samples stored at 40 °C for 6 months, 40 °C and 75% RH for 6 months, and in excess of ICH light conditions) or end of life samples (past expiry) instead of stressed samples to establish peak purity of the active ingredient and specificity for significant degradation products. Forced degraded samples may contain degradation products that interfere with the assay or are not well resolved from other critical analytes but that are, in practice, not observed at significant levels during the course of the formal stability program.
8. FDA, "International Conference on Harmonization: Guideline on Impurities in New Drug Substances," *Federal Register* (Notices) **61** (3), 371–376 (4 January 1996) (ICH Q3A) and FDA, "International Conference on Harmonization: Draft Revised Guidance on Impurities in New Drug Substances," *Federal Register* (Notices) **65** (140), 45085–45090 (20 July 2000) [ICH Q3A(R)]. ICH Q3A(R) currently is at Step 3.
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11. FDA, "International Conference on Harmonization: Guideline on the Validation of Analytical Procedures: Methodology, Availability, Notice," *Federal Register* **62** (96), 27463–27467 (19 May 1997).

12. K.A. Conners, G.L. Amidon, and V.L. Stella, *Chemical Stability of Pharmaceuticals* (Wiley and Sons, New York, New York, 2d Ed., 1986), p. 19 and Reference 3.
13. FDA, "International Conference on Harmonization: Guidance on Q6A Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances," *Federal Register* (Notices) **65** (251), 83041–83063 (29 December 2000) (ICH Q6A) and FDA, "International Conference on Harmonization: Draft Guidance on Q6A Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances," *Federal Register* (Notices) **62** (227), 62889–62910 (25 November 1997) (ICH Q6A). **PT**