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ScienceDirect
Advanced Drug Delivery Reviews 59 (2007) 29–37
Advanced DRUG DELIVERY Reviews
www.elsevier.com/locate/addr

The role of degradant profiling in active pharmaceutical ingredients and drug products ☆

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Received 2 February 2006; accepted 25 October 2006
Available online 15 November 2006

Abstract

Forced degradation studies are used to facilitate the development of analytical methodology, to gain a better understanding of active pharmaceutical ingredient (API) and drug product (DP) stability, and to provide information about degradation pathways and degradation products. In order to fulfill development and regulatory needs, this publication provides a roadmap for when and how to perform studies, helpful tools in designing rugged scientific studies, and guidance on how to record and communicate results.
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Keywords: Forced degradation; Oxidation; Acid/base hydrolysis; Thermal; Humidity; Stability-indicating; CAMEO

Contents

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Pharmaceutical Impurities: Analytical, Toxicological and Regulatory Perspectives".

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1. Regulatory requirements

From a regulatory perspective, forced degradation studies provide data to support the following:

- identification of possible degradants
- degradation pathways and intrinsic stability of the drug molecule
- validation of stability indicating analytical procedures.

Issues addressed in regulatory guidances include:

- Forced degradation studies are typically carried out using one batch of material.
- Forced degradation conditions are more severe than accelerated stability testing such as >50 °C; ≥75% relative humidity; in excess of ICH light conditions; high and low pH, oxidation, etc.
- Photostability should be an integral part of forced degradation study design [1].
- Degradation products that do not form in accelerated or long-term stability may not have to be isolated or have their structure determined.
- Mass balance should be considered.

Issues not specifically addressed in regulatory guidance:

- Exact experimental conditions for forced degradation studies (temperatures, duration, extent of degradation, etc.) are not specified.
- Experimental design is left to the applicant's discretion.

There are guidances available from the FDA as well as from private industry on regulatory requirements for IND and NDA filings [2]. This paper gives a global perspective on regulatory requirements (e.g., USA, Europe and Japan) based on current regulations and guidances.

1.1. Summary of requirements at the IND stage

The reporting of forced degradation study conditions or results is not required in Phase 1 or 2 INDs. However, preliminary studies are encouraged to facilitate the development of stability indicating methodology. It is recommended that be conducted as early in the development of API and DP as possible. Studies can be conducted on the API and developmental formulations to examine for degradation by thermolysis, hydrolysis, oxidation, and photolysis to evaluate the potential chemical behavior of the active. A draft guidance document suggests that results of one-time forced degradation studies should be included in Phase 3 INDs [3].

1.2. Summary of requirements for marketing application

Completed studies of the degradation of the API and DP are required at the NDA stage, including isolation and/or characterization of significant degradation products and a full written account of the degradation studies performed [4].

Requirements at the time of registration include [1]:

- Forced degradation products should be accurately characterized and the reaction kinetics established.
- Structural elucidation of degradation products should be attempted, even if not successful, should be referenced in the NDA.
- Mass balance should be determined or at least attempted.
- Main band peak purity should be confirmed.
- Any degradants present in ICH stability samples which are greater than the identification threshold should be isolated and identified.

Information from these studies should be referenced in the filing and should provide:

- degradation pathways of the API, alone and in DP
- discussion of any possible polymorphic or enantiomeric substances, and
- differentiation between drug related degradation and excipient interferences.

The tables in Appendices A and B outline general protocol of tests and conditions recommended for regulatory submissions.

2. Forced degradation timing and strategy

The requirements for forced degradation testing depend on

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For example, pre-clinical through phase 2 project needs dictate intense method development, and the rate of compound attrition is high. Therefore, when developing a rational study design, forced degradation deliverables should be focused on method development activities, and not isolation and identification of degradants. As a compound progresses into later phase 2 through registration, method development activities center on optimization. The focus of stress testing should be directed to characterization and elucidation of degradants. Table 1 and Fig. 1 below outline the timing and strategy for carrying out forced degradation experiments.

2.1. Degradation discussion

Degradation background discussion is a critical first step in the process. In these initial discussions, degradation prediction, background knowledge and lessons learned can be shared. The purpose of the discussion is to review stability and degradation mechanisms for API and DP in a team-based environment to be used as a resource tool to aid analysts in performing forced degradation studies. Degradation discussions are held to facilitate meeting milestone deliverables, such as stability indicating methodology. Participants include analysts, process chemists, formulators and discovery representatives. Discussion should be reassessed for API process or salt changes, DP formulation changes as well as line extension efforts.

3. Degradation prediction tools

3.1. CAMEO

CAMEO [5] is a computer program that predicts the products of organic reactions given starting materials, reagents and conditions (see Fig. 1, Step 1: Predict degradants). The analyses cover the following key degradation conditions: basic/nucleophilic, acidic/electrophilic, radical, oxidative/reductive and photochemical as well as mechanistic interpretations of these reactions. In general, the CAMEO algorithms have been designed to give product mixtures that err on predicting more degradation products than actually observed. This is preferable to rules that are too restrictive and reject a

Table 1
Forced degradation timing

Development timing	Actions	Recommendations/rational study design
	Step 1	
Pre-phase 1	• Predict API degradants (Fig. 1, Sec. 1) • Design experimental protocol (Fig. 1, Sec. 2) • Perform experiments (Fig. 1, Sec. 3)	• Focus on experiments resulting in at least 5–20% degradation • If degradation by a certain pathway is not predicted and/or experimental data prove it unlikely, minimal effort should be exerted on that condition
	Step 2	
Pre-phase 1 + 6–12 weeks or after lab experiments are complete	• Assessment of API forced degradation data (Fig. 1, step 4) • Selection of key degradants for analytical method development (Fig. 1, step 6) • Challenge existing analytical methodology (Fig. 1, step 4) • Update degradation database (Fig. 1, step 8)	• Forced degradation data used for rugged method development • Mass balance not required • Track only significant degradants • Track and/or ID significant peaks by RRT and MW (LC/MS) only • Isolation and structure elucidation at this stage not required
➢ Milestone: Initial IND	➢ Provide analytical methodology with data to support stability indicating confidence ➢ Methods supplied with expectation of future method development with change of process and/or salt form	
	Step 3	
Formulation development Phase I–Phase II	• Comprehensive forced degradation experiments for DP and API (Fig. 1, steps 2–3) • Review excipient compatibility data (Fig. 1, step 2) • Challenge existing analytical methodology (Fig. 1, step 4–5) • Update key degradation sample set for DP and API (Fig. 1, step 6) • Update degradation database (Fig. 1, step 8)	• Design experiments to highlight process, salt form and/or formulation changes • Mass balance and peak purity assessment as necessary for method development • ID significant peaks by RRT and MW (LC/MS) only unless more work is necessary for RRF determination or project needs
➢Milestone:	➢ Rugged analytical method with high confidence in stability indicating ability, no further method development activities expected	
	Step 4	
Phase III: ICH stability start	• Attempt full characterization of significant degradants (Fig. 1, step 7)	• Isolation, mechanistic understanding and structure elucidation as required
Phase III: ICH stability start	• Update degradation database (Fig. 1, step 8)	• Significant degradants that are fully characterized should include those seen on real time stability

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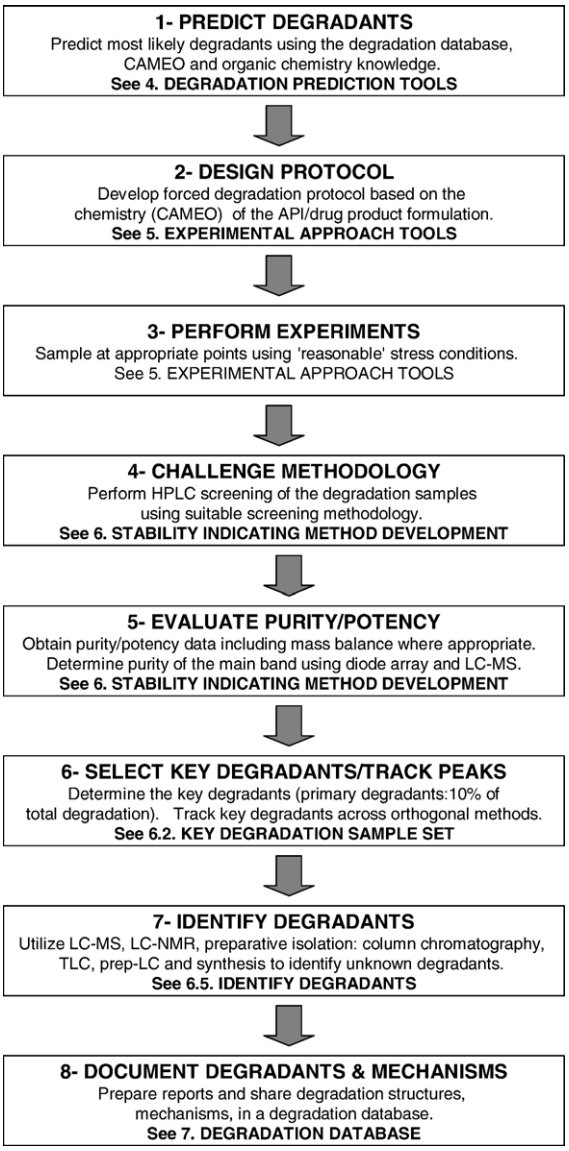

Fig. 1. Forced degradation process flow map — prediction to documentation in a structure searchable global degradation database.

key product observed in actual degradation or ICH stability studies. It is also likely that certain products predicted can undergo further decomposition. Due to these limitations with this prediction program, tracking historical degradation data in terms of functional groups along with CAMEO prediction data provides a more thorough approach to degradation prediction exercises.

4. Experimental approach tools

Forced degradation studies of API and DP include appropriate solid state and solution state stress conditions (e.g. acid/base hydrolysis, heat, oxidation, and light exposure) in accordance with ICH guidelines (Fig. 1, Steps 2 and 3: Design protocol and perform experiments) [1,6]. Forced degradation studies should be conducted whenever a stability indicating method is required

mulations change. The tables in Appendices A and B outline general protocol of tests and conditions that may be used to generate data for regulatory submissions.

4.1. API

The specified stress conditions should result in approximately 5–20% degradation of the API or represent a reasonable maximum condition achievable for the API. The specific conditions (intensity and duration) used will depend on the chemical characteristics of the API. The stressed sample should be compared to the unstressed sample (control) and the appropriate blank. A compound may not necessarily degrade under a given stress condition. No further stressing is advised in these cases [2].

4.1.1. Acid

Example acids include HCl or H_2SO_4 (0.1–1 mol/L solution). Studies should be carried out in the solution state. For certain APIs that are partially soluble or insoluble in the described acidic solution, addition of an appropriate co-solvent, or adjustment of solution pH in the acidic range may be required to achieve dissolution; or the APIs can be run as suspensions [2]. Special attention to the API structure should be paid when choosing the appropriate co-solvent (i.e. do not use alcohols for acidic conditions due to their reactivity). Dimethylsulfoxide, acetic acid and propionic acid are useful under acidic conditions. Additionally, the sample may be heated for a defined time/temperature to accelerate degradation, depending on the API sensitivity to heat.

4.1.2. Base

Example bases include NaOH, LiOH or KOH (0.1–1 mol/L solution). Studies should be carried out in the solution state. For certain APIs which are partially soluble or insoluble in the described basic solution, addition of an appropriate co-solvent, or adjustment of solution pH may be required to achieve dissolution; or the APIs can be run as suspensions. Glyme and 1, 4-dioxane facilitate reactions in basic conditions [7]. Additionally, the sample may be heated for a defined time/temperature to accelerate degradation, depending on the API sensitivity to heat.

4.1.3. Oxidation

Oxidation can be carried out under an oxygen atmosphere or in the presence of peroxides. The use of oxygen is a more realistic model. Free radical initiators may be used to accelerate oxidation. Generally, a free radical initiator and peroxide will produce all primary oxidation degradation products observed on real-time stability. Therefore, free radical and/or hydrogen peroxide conditions are strongly recommended at all stages of development.

For solution state stress conditions, dissolve the API utilizing an appropriate solvent, add 5–20 mol% of a free radical initiator at atmospheric pressure. To increase the solubility of oxygen in the solution, the reaction can be performed in a reaction vessel pressurized at 50–300 psi with molecular oxygen. Additionally, the system is heated to accelerate degradation. The temperature

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For peroxide conditions, hydrogen peroxide reagent (up to 3%) can be used. As previously indicated, the addition of an appropriate co-solvent may be necessary, depending on API solubility. Hydrogen peroxide stress testing can be useful in DP studies where hydrogen peroxide is an impurity in an excipient.

Solid-state stress conditions may be similarly investigated by placing the API (as is) in suitable closed containers filled with an oxygen headspace versus an argon or nitrogen control headspace. Additionally, the sample may be heated for a defined time/temperature to accelerate degradation, depending on the API sensitivity to heat.

For later stage development compounds when more time and effort can be focused on mechanistic understanding, the following oxidation conditions can be applied. The addition of metal ions to solutions of API can indicate whether there is a tendency for the API to be catalytically oxidized. Iron and copper ions are routinely found in APIs and formulation excipients [8]. Transition metal ions can also reduce peroxide to generate hydroxyl radicals in a Fenton-type reaction. In addition, light can also effect oxidation reactions. Light absorbed by a photosensitizer can react with molecular oxygen to form the more reactive singlet oxygen species.

4.1.4. Thermal/humidity

Solid state stability can be evaluated utilizing accelerated storage temperatures in general greater than 50 °C and >75% relative humidity. The duration of exposure is dependent on the API sensitivity. If the forced degradation thermal/humidity conditions produce a phase change, it is recommended to also run thermal/humidity conditions below the critical thermal/humidity that produces the phase change.

Arrhenius kinetics may be used to establish an appropriate temperature and maximum duration of thermal degradation studies. Using an appropriate assumption of activation energy, the duration of controlled room temperature storage that is simulated by the study can be estimated. The table in Appendix C provides a guide to that conversion. In general, an activation energy assumption of 15 kcal/mol is recommended. In certain preclinical through phase 2 studies, an activation energy assumption between the recommended 15 kcal/mol assumption and an aggressive assumption of 18 kcal/mol might be appropriate. In studies where particular concerns exist, an activation energy assumption between the recommended 15 kcal/mol assumption and a conservative assumption of 12 kcal/mol might be appropriate. Deviation from Arrhenius kinetics is increasingly expected above 70–80 °C, and the impact of this should be considered during experimental design.

4.1.5. Photostability

Perform studies in accordance with ICH photostability guidelines [9]. Option 1 and/or Option 2 conditions can be used. According to the ICH guideline, "the design of the forced degradation experiments is left to the applicant's discretion although the exposure levels should be justified. The recommended exposures for confirmatory stability studies are an overall illumination of not less than 1.2 million lux hours and an

For forced degradation studies, the samples should be exposed to at least 2× the ICH exposure length to ensure adequate exposure of the sample. For solution studies, acetonitrile is the co-solvent of choice. Methanol can produce more artifact degradation products from methoxy radicals produced from light exposure.

4.2. Drug product

Drug product (DP) degradation cannot be predicted solely from the stability studies of the API in the solid state or solution. The non-active pharmaceutical ingredients can also react with the API or catalyze degradation reactions. Impurities in the excipients can also lead to degradation in the DP not originally observed in the API. For DP formulations, heat, light, and humidity are often used. The DP stress conditions should result in approximately 5–20% degradation of the API or represent a reasonable maximum condition achievable for a given formulation. The specific conditions used will depend on the chemical characteristics of the DP. For a solid DP, key experiments are thermal, humidity, photostability and oxidation, if applicable. For solution formulations, key experiments are thermal, acid/base hydrolysis, oxidation and photostability. It is recommended to compare stressed samples with unstressed samples and an appropriate blank. For DP studies, the blank sample is an appropriate placebo. The stressed placebo sample will provide information about excipient compatibility.

It is advised to take kinetic time points along the reaction pathway for API and DP degradation studies to determine primary degradants and a better understanding of the degradation pathway.

5. Stability-indicating method development

A stability-indicating method is defined as an analytical method that accurately quantitates the active ingredients without interference from degradation products, process impurities, excipients, or other potential impurities. A method that accurately quantitates significant degradants may also be considered stability-indicating. A proactive approach to developing a stability indicating HPLC method should involve forced degradation at the early stages of development with the key degradation samples used in the method development process (Fig. 1, Step 4: Challenge methodology). Forced degradation should be the first step in method development. If forced degradation studies are performed early, method development and identification of primary degradation products and unknown impurities can be run in parallel. Using this process, a validated HPLC analytical assay, mechanisms of degradation, and the impurity/degradant information for filing can all be generated without delays in the project timeline.

5.1. Mass balance

Mass balance is defined in the 1999 ICH Guidelines as "*adding together the assay value and levels of degradation products to see how closely these add up to 100 percent of the initial value, with due consideration of the margin of analytical*

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