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Special Topic Commentary

Pharmaceutical Forced Degradation (Stress Testing) Endpoints: A Scientific Rationale and Industry Perspective

Todd Zelesky[a,*], Steven W. Baertschi[b], Chris Foti[c,*], Leonardo R. Allain[d], Steven Hostyn[e], Juçara Ribeiro Franca[f], Yi Li[c], Stacey Marden[g], Shikhar Mohan[c], Mariah Ultramari[h], Zongyun Huang[i], Neal Adams[j], John M. Campbell[k], Patrick J. Jansen[l], Dorina Kotoni[m], Christian Laue[n]

[a] *Analytical Research & Development, Pfizer Inc., Eastern Point Road, Groton, CT 06340, USA*
[b] *Baertschi Consulting LLC, Carmel, IN 46033, USA*
[c] *Analytical Development and Operations, Gilead Sciences Inc., Foster City, California, USA*
[d] *Merck & Co., Inc., Rahway, NJ, USA*
[e] *Predictive Analytics & Stability Sciences CoE, Janssen Pharmaceutica, Johnson & Johnson, Beerse, Belgium*
[f] *Brazilian Health Regulatory Agency (ANVISA), Brasilia, DF, Brazil*
[g] *Advanced Drug Delivery, Pharmaceutical Sciences, R&D, AstraZeneca, Boston, MA, USA*
[h] *Spektra Soluções Científico-Regulatórias Ltda, São Paulo, Brazil*
[i] *Bristol-Myers Squibb Company, 1 Squibb Drive, New Brunswick, NJ 08901, USA*
[j] *Pfizer, Scientific and Laboratory Services – Analytical Sciences, Pfizer Inc., 7000 Portage Road, Kalamazoo, MI 49001, USA*
[k] *Analytical Development, GSK, Upper Providence, PA 19426, USA*
[l] *Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285, USA*
[m] *Chemical & Analytical Development, Novartis Pharma AG, Basel, Switzerland*
[n] *Chemical & Pharmaceutical Development, Merck Healthcare KGaA, Darmstadt, Germany*

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ABSTRACT

Forced degradation (i.e., stress testing) of small molecule drug substances and products is a critical part of the drug development process, providing insight into the intrinsic stability of a drug that is foundational to the development and validation of stability-indicating analytical methods. There is a lack of clarity in the scientific literature and regulatory guidance as to what constitutes an "appropriate" endpoint to a set of stress experiments. That is, there is no clear agreement regarding how to determine if a sample has been sufficiently stressed. Notably, it is unclear what represents a suitable justification for declaring a drug substance (DS) or drug product (DP) "stable" to a specific forced degradation condition. To address these concerns and to ensure all pharmaceutically-relevant, potential degradation pathways have been suitably evaluated, we introduce a two-endpoint classification designation supported by experimental data. These two endpoints are 1) a % total degradation target outcome (e.g., for "reactive" drugs) or, 2) a specified amount of stress, even in the absence of any degradation (e.g., for "stable" drugs). These recommended endpoints are based on a review of the scientific literature, regulatory guidance, and a forced degradation data set from ten global pharmaceutical companies. The experimental data set, derived from the Campbell et al. (2022) benchmarking study,[1] provides justification for the recommendations. Herein we provide a single source reference for small molecule DS and DP forced degradation stress conditions and endpoint best practices to support regulatory submissions (e.g., marketing applications). Application of these forced degradation conditions and endpoints, as part of a well-designed, comprehensive and a sufficiently rigorous study plan that includes both the DS and DP, provides comprehensive coverage of pharmaceutically-relevant degradation and avoids unreasonably extreme stress conditions and drastic endpoint recommendations sometimes found in the literature.

Introduction

Forced degradation (i.e., stress testing) has long been recognized as a critical part of the drug development process, providing insight into

* Corresponding authors.
E-mail addresses: todd.c.zelesky@pfizer.com (T. Zelesky), chris.foti@gilead.com (C. Foti).

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the intrinsic stability of a drug, and being foundational for the development and validation of stability-indicating analytical methods. The aim of the forced degradation study is to induce within a short period of time, degradation pathways that have the potential to occur during manufacture, long-term storage, distribution, and use.[2,3] To achieve this aim, the experimental conditions need to be designed appropriately to determine the degradation pathways that are pharmaceutically relevant. A critical aspect of designing forced degradation experiments is defining the severity and exposure time for each condition together with providing meaningful "endpoints". A science based forced degradation endpoint is defined as application of sufficient amount of stress to ensure all pharmaceutically-relevant potential degradation pathways/products have been suitably evaluated.

Setting appropriate endpoints can be challenging. Each drug substance and its formulated product can undergo different degradation rates and pathways as a function of the stress conditions. Based on molecular structure and the associated physicochemical properties, some drugs may be stable or reactive to forced degradation stress conditions. Excessive stressing, resulting from inappropriately chosen conditions and endpoints, increases the risk of generating degradation products that are not relevant, i.e., different from those that may be observed on storage, distribution, or use. Such stressing would lead to misunderstanding the relevant drug degradation pathways, resulting in "overengineering" the corresponding stability-indicating methods. Conversely, insufficient stress would provide an underestimate of the range of potential degradation products that could be formed and could lead to degradation products detected in formal stability studies that were not observed in stress testing studies. To overcome these challenges, herein we provide recommendations for a well-designed forced degradation study plan with due consideration of relevant conditions and appropriate endpoints based on a review of the scientific literature, regulatory guidance, and a detailed analysis of a forced degradation data set from ten global pharmaceutical companies.

Historical Context

The topic of forced degradation is well-represented in the scientific literature across numerous publications over the past twenty years,[3-8] including two editions of a book dedicated to the subject (2005 and 2011)[9,10] and a stress testing benchmarking study in 2003 surveying twenty pharmaceutical companies on their internal forced degradation study best practices.[11] Historically, a wide diversity of stress conditions have been used to establish the potential degradation products for drugs.[2,12] For example, such conditions were described in various chapters in a book by Grimm and Thomae in 1985[13] and in the 1972-1991 book series "Analytical Profiles of Drug Substances".[14] In 2000, Singh and Bakshi documented this diversity in a guidance paper, and proposed specific conditions along with decision trees for performing hydrolytic, oxidative and photolytic stress studies.[12] For each of these conditions, a stepwise increase in the severity of the applied stress was recommended in order to reach an endpoint for a level of degradation recommended to be between 20 and 80% degradation. Based upon these predefined conditions and endpoints, a condition-based stability classification system (6 classes ranging from extremely labile to practically stable or photostable versus photolabile) was proposed. In 2002, the Pharmaceutical Research Manufacturers Association (PhRMA) developed a guidance for conducting forced degradation studies, asserting that "sufficient exposure is achieved when the DS has degraded ~10% from its initial amount or after exposure in excess of the energy provided by accelerated storage over 6 months, whichever comes first".[4] In 2003, Alsante et al. published a benchmarking study of 20 pharmaceutical companies, capturing the diversity of approaches to stress conditions, endpoints, procedures, etc.[11] Two years later in 2005, Baertschi published the first book dedicated to the topic of pharmaceutical stress testing, and discussed the significant variability of historical approaches used for forced degradation conditions, reagents, and endpoints in Chapter 1.[15] The book also provided scientific foundations for specific stress conditions, endpoints, as well as practical recommendations for execution of stress studies. In 2007, Alsante et al., building on these recommendations, provided additional practical recommendations for conducting pharmaceutical forced degradation studies.[5] In 2011, a 2nd edition of the pharmaceutical stress testing book was published, with detailed recommendations, and scientific rationale for the choice of stress conditions with limited discussion of endpoints.[2]

Thermal/Humidity Stress

The importance of elevated temperature and humidity have long been recognized as critical aspects of forced degradation studies, as described in some detail by Baertschi et al.[2] In 1964 Kennon described the changes in rates of degradation of pharmaceutical products resulting from increasing temperatures from room temperature to 85 °C.[16] The ICH Q1A Stability guideline[17] suggests studying the effect of temperature in 10 °C increments (e.g., 50 °C, 60 °C) above the accelerated temperature test condition along with elevated humidity (e.g., 75% relative humidity (RH) or greater). It is not clear why the guideline suggests multiple temperatures in 10 °C increments, but it could be related to the potential of using multiple temperatures to establish Arrhenius-type relationships, along with the possibility that apparent degradation pathways can change as a result of increasing temperature, especially in the solid state (presumably due to potential phase changes). To minimize the risk of such changes, it has been suggested to, in general, limit the highest temperature to ~70 °C.[2] With regard to the effects of humidity, the use of 75% RH for the elevated humidity condition has become an informal industry standard for evaluation of the effects of elevated humidity; storage conditions for low humidity values have been recommended to be 20% RH or lower.[2] As mentioned in the Historical Context Section above, PhRMA guidance recommended that the thermal stress conditions should exceed the kinetic equivalent to storage at accelerated conditions (e.g., 40 °C for 6 months for a room temperature storage product); this recommendation is affirmed by Baertschi et al.[2] and Kleinman.[18] As discussed in some detail by Kleinman and Baertschi, it is relatively straightforward to estimate the kinetic equivalent by assuming the energy of activation (E_a) recommended by USP for mean kinetic temperature calculations (i.e., 83.14 kJ/mol, 19.87 kcal/mol).[19]

Acid/Base Stress

Hydrolysis has long been asserted to be a common pathway for drug degradation.[20] Historically, conditions employed for forced degradation studies have varied dramatically, from dilute solutions of acids and bases at room temperature to concentrated solutions at refluxing temperatures.[2,12,21] The ICH Stability guideline simply suggests evaluation of the susceptibility to hydrolysis "across a wide range of pH values when in solution or suspension".[17] Baertschi et al. have asserted that a pH of 1 (e.g., 0.1 N HCl) is a reasonable pH limit to explore the acid-catalyzed degradation of drugs.[2] For the upper end of pH concerns, analytical methodologies / handling has led to a general industry alignment around the pH of 13 (e.g., 0.1 N NaOH or KOH). MacFaul et al.[22] observed Arrhenius behavior for 166 drug-like compounds in acid/base solution studies up to 90°C, but degradation profiles sometimes changed at the higher temperatures, illustrating the risk of using high temperatures. In the last decade or so, the pharmaceutical industry has in general aligned around an upper limit of 70-80°C for hydrolysis studies.

Oxidative Stress: Peroxide

Oxidative forced degradation studies have historically focused on exposure to peroxides (a non-radical oxidative process when conducted at room temperature under transition metal free conditions) of different strengths, times, and temperatures.[2,11] Historical studies often used 0.3% (w/w) hydrogen peroxide as the standard concentration, presumably because of the ease of preparation by dilution from standardized 3% (w/w) aqueous reagent solutions, but many variations of strengths, times, and temperatures have been utilized.[2,12] More recently, Boccardi[23] and Baertschi et al.,[2] recommended 0.3-3% (w/w) hydrogen peroxide concentrations with duration of 2-7 days and a limit of 40 °C to avoid the potential for unwanted O-O homolytic bond cleavage to form hydroxyl radicals, which are very strong oxidants that lead to oxidative degradation that is rarely observed in drug formulations. Unfortunately, peroxide-based oxidative stressing misses a significant portion of pharmaceutically-relevant oxidation, namely, radical-mediated autoxidation.

Oxidative Stress: Radical Initiators

While radical-mediated oxidative processes (also known as autoxidation) have long been recognized as relevant to drug oxidative degradation,[24] recommendations for such studies in pharmaceutical forced degradation studies in a systematic way was not proposed until 1992 by Boccardi et al.[25] Boccardi et al. demonstrated that the azo radical initiator AIBN (2,2′-Azobisisobutyronitrile) in acetonitrile at 40°C for 48 hours effectively generated radical-mediated oxidative degradation products of tetrazepam that were not observed using peroxide stress but yet were observed as actual degradation products in tablets of the same drug. Almost a decade later, the importance of radical oxidation to drug degradation was discussed in detail by Hovorka et al.[26] and later by Waterman et al.[27] However, by the time the first benchmarking study was published in 2003,[11] only 5 out of 19 companies used radical stress as part of their forced degradation studies. Even as late as 2005, Klick et al.[7] recommended radical forced degradation to be executed only on “a case-by-case approach”.

In 2005, Boccardi provided a thorough discussion of oxidative degradation in the context of drug degradation,[23] including specific conditions, for the use of AIBN in forced degradation studies (i.e., concentration of ~5 mM, temperature of 40 °C, duration of 2 days, solvent of acetonitrile and/or methanol with up to 20% water). Boccardi also mentioned that the use of azo radical initiators other than AIBN can be used, with adjustments for the temperature based on the specific half-life in comparison to AIBN. For example, he mentioned that VAZO52 (i.e., 2,2′-Azobis(2,4-dimethylvaleronitrile), the initiator used by company C in Fig. 3(d)), at the same concentration, can be used at 25 °C with a similar timescale as AIBN at 40 °C. In 2006, the experimental conditions to improve the azo radical initiator-based autoxidation stress test experiment were refined in a series of publications. Nelson et al. found that ambient air can provide sufficient oxygen at low initiator concentration, demonstrating that high-oxygen atmospheres (e.g., 100% O_2 headspace) were not needed for such studies.[28] In the next publication, Nelson et al. investigated the effect of various solvents in the autoxidation of cumene using AIBN.[29] The authors discovered that unwanted alkoxy radicals can be formed in the autoxidation experiment (leading to the formation of non-pharmaceutically-relevant oxidation products), and that the addition of 3-10% v/v methanol can scavenge these unwanted alkoxy radicals. In 2013, Watkins et al. built further on these findings and advocated the addition of ca. 10 volume% methanol to the reaction medium and to use a sufficiently high radical initiator/drug ratio (>100 mol%), to prevent analytical artifacts.[30] A thorough discussion of all of these aspects is provided by Harmon and Boccardi in their book chapter on “Oxidative Susceptibility Testing”.[31] In 2015, Nefliu et al. reported on the formation of α-aminonitrile and urea artifacts upon the use of azo radical initiators in combination with primary and secondary amines (with or without the addition of methanol).[32] Other radical initiation systems based on non-azo radical initiators have also been proposed.[33,34] Taken together, it is clear that the inclusion of radical-mediated oxidative stress conditions is an important aspect of evaluating the potential oxidative degradation pathways of a drug molecule, and that recommended conditions are well-described in the literature.

Oxidative Stress: Transition Metals

The use of transition metals to induce oxidative degradation has long been known. One of the first examples in the literature of the use of metal ions to induce the formation of degradation products of a drug traces back to 1972, when levarterenol bitartrate was shown to degrade using Cu^{2+}.[35] In 1992, Boccardi et al. used $FeCl_3$ and $CuSO_4$ in acetonitrile to degrade electron rich benzodiazepine derivative tetrazepam.[25] In 2002, Waterman et al. recommended the use of <100 ppm of $FeCl_3$ and $CuCl_2$ for forced degradation of liquid dosage forms.[27] The benchmarking study of 2003 by Alsante et al. showed that at that time only 3 out of 19 companies were using metal solutions in forced degradation studies.[11] In 2004, Klick at al. recommended metal forced degradation to be executed on “a case-by-case approach”.[7] In 2005, Boccardi asserted that transition metals can be an important probe for detecting the susceptibility of drug molecules to oxidation.[23] Transition metal ions can initiate autoxidation via different mechanisms than radical initiators (e.g., selective complexation of specific moieties and initiation of electron transfer reactions, reduction of adventitious peroxides to radicals, activation of molecular oxygen by complexation, etc.). While acknowledging the potential utility for other transition metals such as Mn^{2+}, Boccardi, suggested the use of Fe^{3+} and Cu^{2+}, describing them as “discriminating agents” that do not always yield the same oxidative degradation results, and that drug substances that are not oxidized by either metal ion are “normally inert” to transition metal-catalyzed oxidation. In 2011, Harmon and Boccardi provided additional detail on the use of transition metals for forced degradation studies,[31] and specific conditions for such studies were recommended by Baertschi et al.[2] In 2012, Baertschi reported a study of 15 drugs for which complete forced degradation and formal stability results were available;[36] this study showed that of the 31 oxidative degradation products formed during forced degradation studies using peroxide and radical initiators, six of these products were also formed by Fe^{3+} and Cu^{2+} stressing. Interestingly, Fe^{3+} / Cu^{2+} stressing also formed an additional product that was a unique degradation product (formed under no other stress conditions) that was also relevant in that it was observed in accelerated and long-term stability of the drug product.[36] Although transition metals can induce other pathways (e.g., via complexation, hydrolytic catalysis of specific sites in the drug molecule, etc.), Harmon discussed four general modes of metal ion catalysis of oxidation.[31] In general, the use of transition metal ions is considered to induce single electron transfer (SET) oxidative degradation pathways and/or to decompose trace hydroperoxides to radicals. One noteworthy example is described by Nanda et al., where Fe^{3+} ions were shown to induce a pharmaceutically-relevant SET pathway (i.e., a pathway observed in the formulated product on stability), specifically benzylic oxidation of certain aromatic drug molecules via the formation of a π-stabilized aromatic radical cation;[37] surprisingly, peroxy radicals were not able to induce this degradation pathway. Finally, it should be mentioned that WHO guidelines (TRS 929, Annex 5, discussed in Section 1.3) discuss the use of metal ions (Fe^{3+} or Cu^{2+} at 0.05 M for 1-10 days) as an “optional” test. ANVISA[38] discussed the use of metal ions (“usually Fe^{3+} or Cu^{2+} solutions”) for 1 day, and that the sample temperature and overall concentration of the metals is not critical.

Other Stress and Oxidative Conditions

While the oxidative stress conditions outlined above can be considered comprehensive for the vast majority of oxidative drug degradation examples, oxidative degradation from other reactive oxygen species (e.g., hydroxyl radicals, singlet oxygen, ozone, superoxide radical anion) can also occur, although much less frequently. For example, Baertschi et al.[2] discussed the use of other oxidative reagents / techniques (e.g., peracetic acid, meta-chloroperoxybenzoic acid, potassium monoperoxysulfate, singlet oxygen production, sodium hypochlorite, potassium permanganate, and Fenton's reagent) as "investigative oxidative stress tests". Stressing in the presence of N-methylpyrrolidone and oxygen has been shown to induce pharmaceutically-relevant oxidative degradation via peroxide, radical, and singlet oxygen pathways in a single stress condition and is used by some companies for routine oxidative stress testing.[34,39] Additionally, urea-hydrogen peroxide undergoes solid-state decomposition releasing hydrogen peroxide vapor at elevated temperatures and can be used as a reagent for targeted solid-state oxidative stress.[40,41] The use of such oxidative stress reagents can often be useful to produce specific oxidative degradation products more selectively or more rapidly (e.g., for method development or structure elucidation purposes).

Additional stress condition options include mechanoactivation techniques (e.g., ball milling, cryomilling). Resultant crystal defects, crystalline to amorphous conversions, reduction in particle size, etc. can impact degradation kinetics increasing degradation rates; however, additional studies are needed to fully understand applicability of this relatively new field in the pharmaceutical industry.[42-44]

Photostress

Lastly, the historical development of photostability forced degradation is best described by Piechocki,[45] while the diversity of photostressing of drugs is described by Baertschi and Reynolds[46] (and references therein). It is clear that ICH Q1B (published in Nov 1996)[47] provided significant harmonization of light sources (with two Options), relevant irradiation wavelengths, total photo-exposure recommendation, and sample presentation for both DS and DP. A thorough overview of ICH Q1B is provided by Baertschi et al.[48,49] and practical aspects of photostability stress testing were discussed in detail by Clapham et al.[50] ICH Q1B describes the amount of photoexposure needed for both "forced degradation" studies and for "confirmatory studies". Confirmatory studies involve testing under conditions designed to mimic typical photoexposures during manufacturing, storage and distribution, and thus are part of formal stability studies; the minimum recommended photoexposure for confirmatory studies is 200 W-h/m^2 in the UV range (320-400 nm) and 1.2 million lux-h in the visible range (400-800 nm). Photoexposures for forced degradation studies, for photostable compounds, are "left to the applicant's discretion", although since the purpose is to understand the intrinsic photostability of the drug compound, the total photoexposure (direct exposure without any packaging protection) would be expected to exceed the confirmatory exposure (typically 2-5X, with a minimum of 2X ICH).[50] With regard to sample presentation in such photo-exposures and practical applications (i.e., sample preparation, sample positioning, effect of heating and humidity), these topics have been discussed in more detail elsewhere.[48-52]

Overall, the historical discussion above shows that the conditions for forced degradation have evolved over the last few decades, leading to a reasonable amount of harmonization that has been vetted in the scientific literature and incorporated into industry practices. The evolution of forced degradation conditions has also informed the development of regulatory guidance, as discussed below.

Regulatory Guidance Review

Although stress testing (i.e., forced degradation) is mentioned in several regulatory guidelines, little information regarding specific endpoints is defined in those guidelines. A brief overview of regulatory requirements for forced degradation endpoint guidelines issued by ICH, WHO, FDA, EMA and ANVISA is presented below.

"Stress testing" is mentioned in ICH Q1A(R2),[17] Q1B,[47] Q2(R1),[53] Q3A(R2),[54] Q3B(R2),[55] and M7 (R1).[56] ICH Q1A(R2) includes some notions of temperatures (e.g., 50°C, 60°C, etc.) and humidity (e.g., 75% RH or greater) and where appropriate, oxidation and photolysis. Such studies should also evaluate the susceptibility of the DS to hydrolysis across a wide range of pH values when in solution or suspension. Specific endpoints are not mentioned in the guidelines. An accurate reading of ICH Q1A(R2) and Q1B gives the understanding that photolytic stress tests should apply a higher photoexposure than those used in confirmatory studies, depending on whether "extensive decomposition occurs".

WHO guideline TRS 1010 - Annex 10 (Stability testing of active pharmaceutical ingredients and finished pharmaceutical products)[57] covers updated recommendations regarding stress testing. This guideline includes the information already found in ICH Q1A(R2) regarding stress testing (see above). Also, it is recommended to test conditions that causes degradation to some extent (typically 10–30% loss of DS as determined by assay when compared with non-degraded DS). The guideline also states that a DS can be considered stable under a particular stress condition if no degradation products are found after testing for 10 days. In such cases, the stress conditions employed should be justified. In an earlier WHO guideline, specific endpoints for each stress condition where described (TRS 929, Annex 5). However, in the updated versions of WHO guidelines, that information was removed.

The document "Guideline on stability testing: stability testing of existing active substances and related finished products" issued by EMA (CPMP/QWP/122/02, rev 1 corr) includes the same information found in ICH Q1A(R2) regarding thermal, humidity, and solution stress testing (see above). From this guideline, it is understood that photolytic stress tests should apply the conditions described in ICH Q1B for forced degradation. No specific stress testing endpoint guidance could be found in regulatory guidelines issued by the FDA.

Detailed information regarding "forced degradation" studies for (drug products) DPs is described in the ANVISA Regulation and associated Guidelines. The Resolution of the Collegiate Board of Directors (RDC) No. 53,[38] of December 4, 2015, prescribes that the studies should promote degradation greater than 10% (ten percent) and less than that which would lead to complete degradation of the sample. In tests where degradation is less than 10% (ten percent), the company must provide a technical justification. Guideline No. 4/2015[58] establishes that it is possible to achieve <10% degradation in some of the stress conditions, provided that "the company demonstrates that the condition used is compatible with the maximum recommended in scientific literature". Moreover, the same Guideline states a more specific endpoint for oxidation mediated by transition metals by informing that "sample temperature or variation in their concentration is not considered relevant" for this stress condition and establishing that the test should be stopped if no degradation is observed after 1 day. Forced degradation of the DS is covered by (RDC) No. 318, of November 6, 2019. The regulation states that the forced degradation should promote a degradation extent enough to "evaluate the degradation products" and less than that which would lead to "excessive and complete degradation" of the DS sample.

It should be noted that according to the FDA, DP stress testing may not be necessary when the routes of degradation and the suitability of the analytical procedures can be determined through use of available data. For well-known DPs, waiving forced degradation could be

proposed when there is available data from stress testing of the DS, reference materials for process impurities and degradants are available, and data from accelerated and long-term studies on the DS and DP are presented. This concept is particularly useful when the analytical method of a mature DP needs to be updated.[59]

From all guidelines listed above, one can conclude that the main goal of the regulatory bodies is to assure that companies have investigated the intrinsic stability of their products, providing the ability to characterize the degradation profile of their products using validated stability-indicating methods. Because DS and DP endpoints are often influenced by the unique physicochemical properties of the DS, DP excipient properties, and DP type (e.g. solid, liquid, semi-solid, etc.), details of stress testing endpoints are undefined in regulatory guidelines and therefore have become company- and product-specific.

Pharmaceutically-Relevant Stress Conditions and Well-Designed Study Plan

Forced degradation studies should target all the stress conditions discussed above and specifics of the scope and design are dependent on the phase of development. These studies are not necessarily a one-time event, but rather can evolve over the course of the drug development lifecycle from preclinical to clinical development to marketing application, as needed. If a study is well-designed, a knowledge space is generated that provides an opportunity to (1) identify potential degradation products that may be reasonably expected to form during long-term storage conditions (aka, potential degradation profile); (2) develop appropriate analytical methodology to resolve, detect, and quantify degradation products; (3) de-risk the potential for unexpected products to appear later in development; and (4) guide DP formulation development from a stability perspective.

In addition to experimental results, the forced degradation knowledge space may include using *in silico* tools such as Zeneth[60] and scientific judgment to predict potential degradation pathways. This information can be used 1) to optimize the forced degradation study design and approach, and 2) to evaluate the theoretical reactivity risk of a DS with excipients (and excipient-related impurities) being considered in formulation development.

Although a consideration of theoretical impurities from *in-silico* modeling and scientific principles can be important in a risk assessment and in developing the drug degradation knowledge space, a well-designed forced degradation study is currently the best approach to predict the degradation products that have the potential to form during long-term stability studies.[60,61] The set of degradation products formed during long-term storage (the actual degradation profile) are, ideally, a subset of those generated during forced degradation experiments (the potential degradation profile).[38] Therefore, it is crucial that the forced degradation study design is tailored to reveal actual degradation products.

A well-designed and comprehensive study of a solid dosage form DP includes solution forced degradation of the DS and solid-state forced degradation of both the DS and DP (Table 1). For solution based DP, thermal stressing would involve heating the solution formulation. It is worth noting the work by Campbell et al.[1] has provided strong evidence that for solid dosage form DPs, forced degradation studies need not include solution-phase stressing of the DP to be comprehensive. Specifics surrounding the experimental conditions and endpoints are discussed in the next sections.

Table 1
Forced degradation study design for solid DPs.

Drug Substance		Drug Product
Solution	Solid State	Solid State
Acid	Thermal (low humidity)	Thermal (low humidity)
Base	Thermal (high humidity)	Thermal (high humidity)
Oxidative: hydrogen peroxide	Photolytic	Photolytic
Oxidative: radical initiator		
Oxidative: transition metals		

Dependency of Stability-Indicating Methods (SIMs) on Forced Degradation Study Plan

Forced degradation studies and the development and validation of stability-indicating methods is (or can be) an iterative process, and the iterative process may need to be repeated throughout the lifecycle of a drug.[5,62] As illustrated in Fig. 1, the acquired knowledge can direct the analytical strategies associated with the development, optimization, and validation of SIMs. These activities include 1) determining the potential degradation profile of the drug[2], 2) a mass balance assessment, and 3) a peak purity assessment of the active component(s). It is the assertion supported by many investigators that partially degraded samples (e.g., 5-20%) from forced degradation studies are both sufficient and appropriate for mass balance assessments.[63] Major degradation products from an individual stress condition would be contributory to the broader potential degradation profile; useful for demonstrating sufficient specificity during SIM validation. A SIM should be suitably selective and able to quantify all "actual" degradation products formed on long-term stability to support a DS retest period and/or DP shelf life. Definition of major degradation products that define the potential degradation profile are discussed in Baertschi et al. (2011) and Kleinman et al.[2,18]

Experimental/Methods Used

Building the Endpoints Data set

Internal forced degradation best practices were surveyed from ten global pharmaceutical companies with the goal of capturing study design details applicable to supporting late phase drug candidates and regulatory submissions (e.g., marketing applications). The survey included small molecule DS and solid dosage form DPs. Companies were asked to provide forced degradation study design details for both solution phase and solid phase forced degradation, and the experimental details for all stress conditions. Solution phase stress conditions surveyed included reagent choice, reagent concentration, temperature, and duration, while solid phase thermal stress conditions included temperature, % relative humidity, and duration, and solid phase photostress included light source and the total photoexposure in both the UV and visible ranges. Participating companies provided both 1) % total degradation target endpoints for drugs they considered reactive and 2) experimental details for each stress condition that defines the maximum amount of chemical stress that would be imparted on the drug to consider the drug stable to that condition.

Data Visualization

The endpoint range plots (Fig. 2) were generated using Tableau 2021.3.7 software. The forced degradation study endpoint ranges were grouped by sample type (solution or solid phase) and stress condition (hydrolysis, oxidation, thermal and light). The numbers shown on the Fig. 2 graphs represent the minimum and maximum % total degradation target outcome under a certain stress condition from a company. Overlaying the endpoint ranges of forced degradation study from all ten pharmaceutical companies provides a direct visualization of similarities and differences in % total degradation target outcomes across the industry.

A collection of maximum amounts of stress endpoint imparted to DS and/or DP were plotted in 3D bar plots using Microsoft Excel

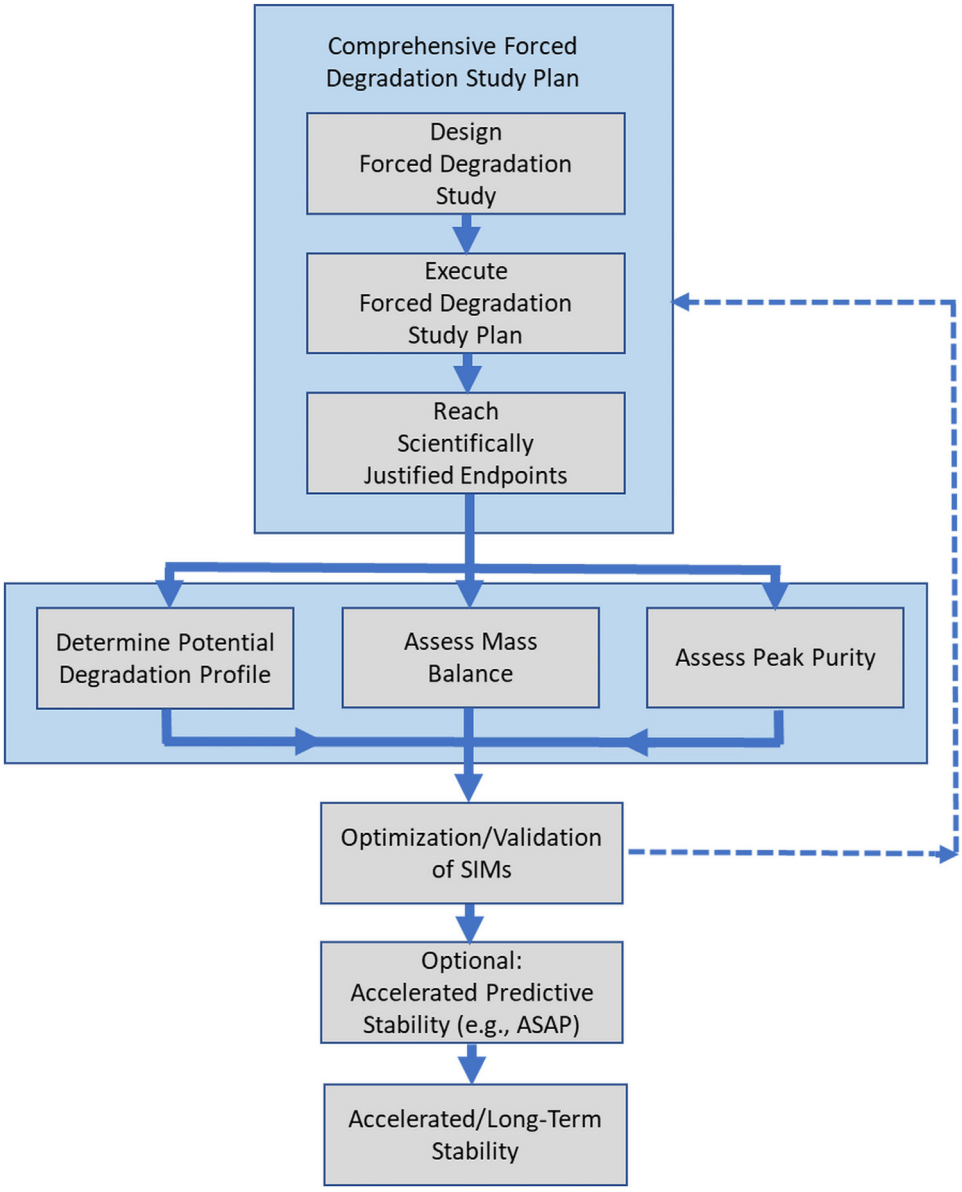

Figure 1. A comprehensive forced degradation study plan, including appropriate endpoints, optimization, and validation of stability-indicating methods (SIMs).

(Fig. 3). Three parameters were used to rank the endpoints from ten companies in ascending order: temperature, stress duration in days, and a third condition-specific parameter that helps further describe the intensity of stress imparted. This third parameter was either reagent concentration for oxidation/hydrolysis solution phase or % relative humidity for thermal solid phase forced degradation. These plots provide a comprehensive view of the stress conditions currently practiced by industry.

The reported Fig. 3(e) %RH values were estimated by calculations (using the average temperatures and %RH conditions when the samples were prepared for storage) to enable plotting. The remaining three companies control relative humidity to either 11% or 20%. Fig. 3 (e) reports ~3%, ~5% and ~7% (estimated uncontrolled %RH) and 11% and 20% (controlled %RH).

The solid phase photostress endpoints plot (Fig. 4) was generated using Tableau software. The shapes are associated with the sample source: circles represent DS and squares represent DP. Photostability endpoints for photostable products are sorted by exposure to an excess of confirmatory recommendations in ICH Q1B (200 W-h/m2 in the UV range (320-400 nm) and 1.2 million lux-h in the visible range (400-800 nm)).

Results and Discussion

Endpoint Classification System

A thorough literature search combined with a detailed study of forced degradation best practices of the ten companies involved contributing to the data set in this paper have led to the development of a science-based two-endpoint classification designation proposal. Stress endpoints can be either the amount of degradation (as measured by loss of parent) in the range of 5-20% (Endpoint 1) (e.g., for "reactive" drugs) or a specified amount of stress, even in the absence of any degradation (Endpoint 2)(e.g., for "stable" drugs), whichever is reached first, without exceeding 20% total degradation. The recommended amount of pharmaceutically-relevant stress (RAPRS) for Endpoint 2 is described and defined in the sections below. This proposal is applicable to small molecule DS and solid dosage form DP

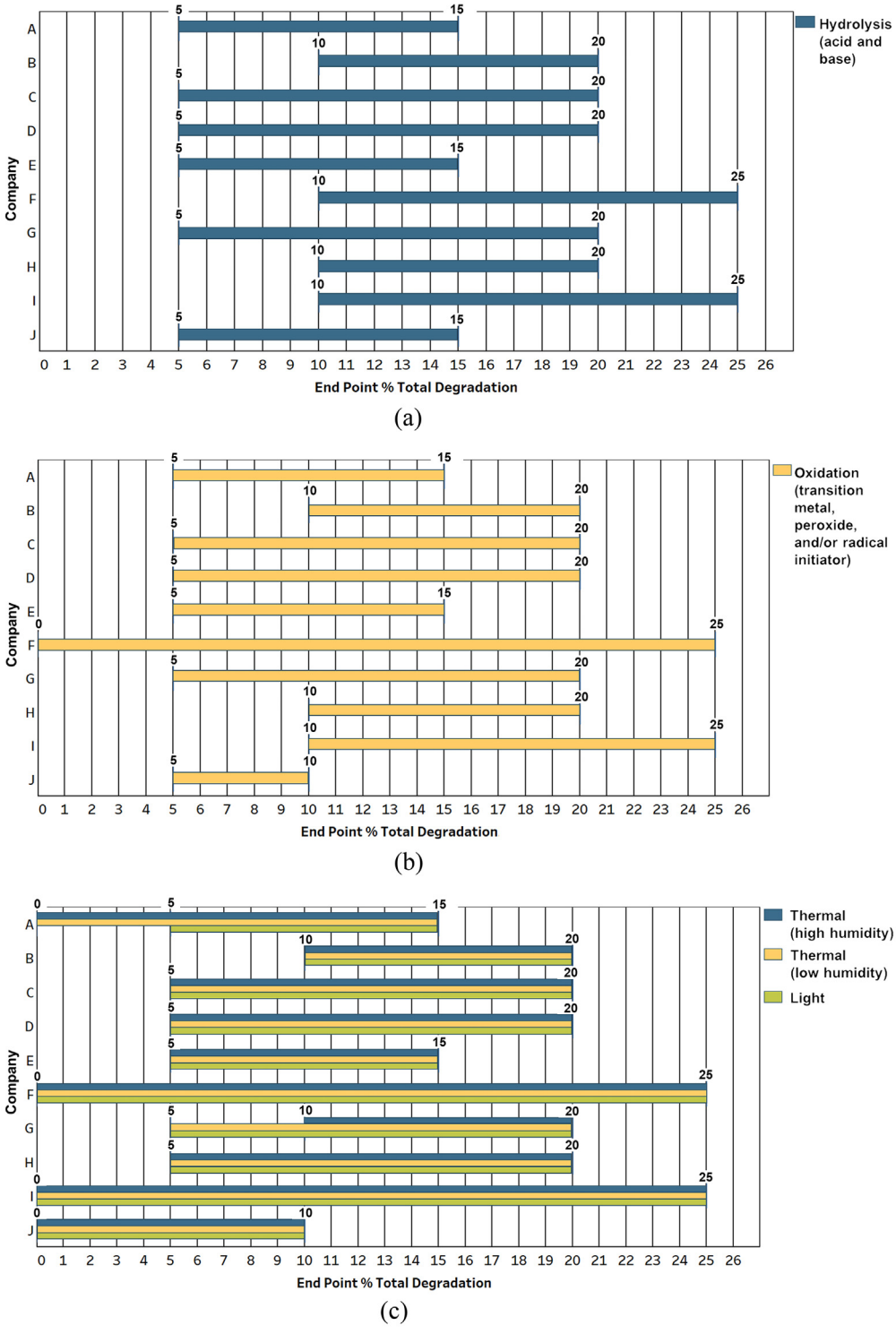

Figure 2. Percent total degradation endpoint from ten global pharmaceutical company internal forced degradation best practices for drugs considered "reactive"; (a) DS solution phase hydrolysis stress, (b) DS solution phase oxidative stress, (c) DS and DP solid phase thermal and photostress.

forced degradation studies intended to support regulatory submissions. For a given stress condition, achieving Endpoint 2 by imparting the recommended amount of stress (i.e., RAPRS) to a drug where not more than 20% total degradation is reached results in a classification of "stable" (0% degradation), "moderately stable" (0 to < 5% degradation) or "reactive" (5-20% degradation). Outcome-based recommendations are tabulated in Tables 2 and 11 and are applicable to both DS (solution and solid phase forced degradation) and solid DPs (solid phase forced degradation).

Data Supporting Proposed Two-Endpoint Classification System

Ten global pharmaceutical companies contributed key data related to internal company forced degradation best practices,

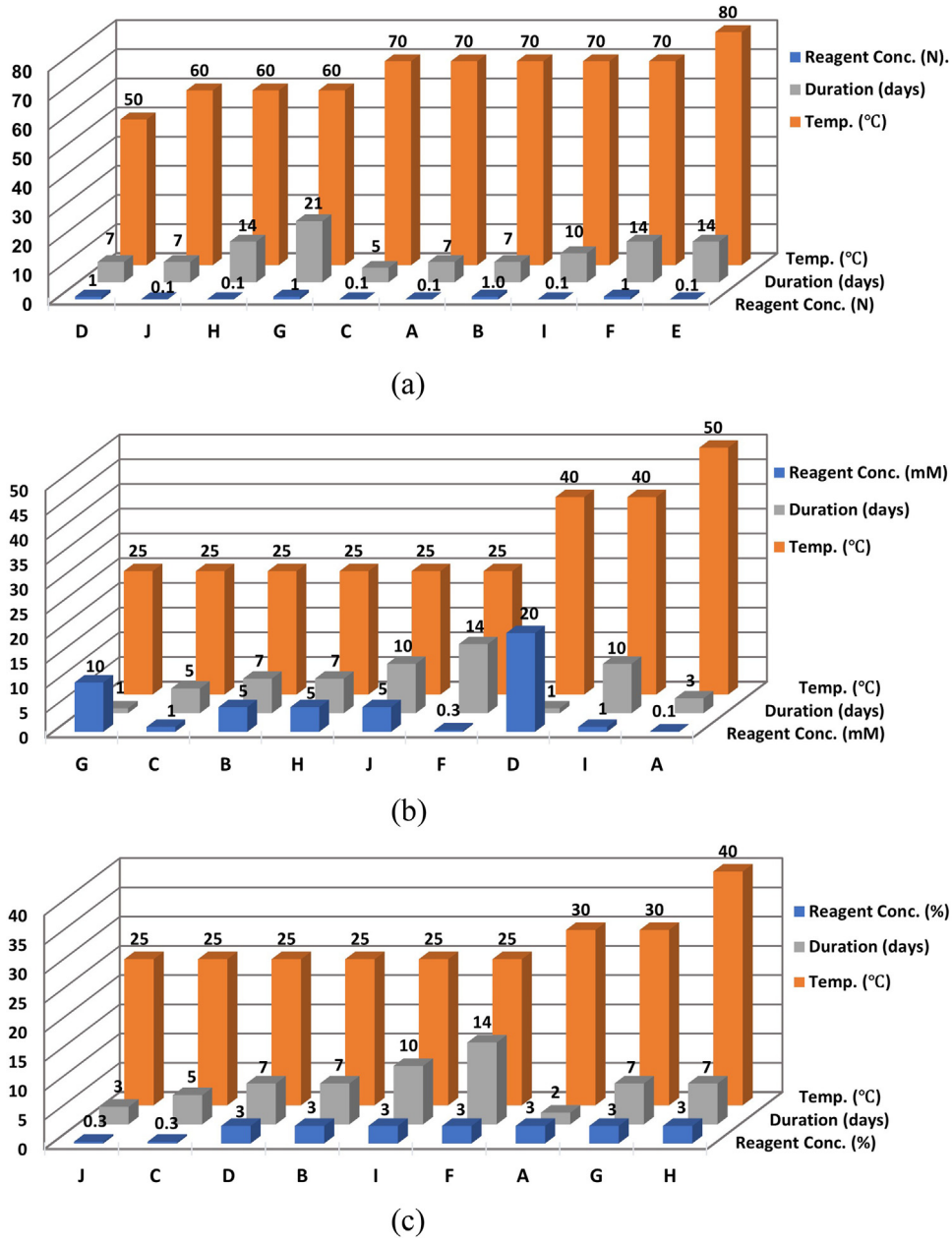

Figure 3. Amount of chemical stress ten global pharmaceutical companies (A-J) impart on a drug to ensure all pharmaceutically-relevant degradation pathways have been identified; (a) DS solution phase hydrolysis stress; Acid (HCl) and Base (NaOH), (b) DS solution phase oxidative stress; transition metals Fe^{3+} and Cu^{2+} salts, (c) DS solution phase oxidative stress; hydrogen peroxide, (d) DS solution phase oxidative stress; radical Initiator, (e) DS and solid DP thermal (Low Humidity) stress, (f) DS and solid DP thermal (High Humidity) stress.

including specific stress conditions and endpoints. The survey data are captured in Figs. 2-4, allowing for direct and rapid data visualization of the similarities and differences across the industry forced degradation endpoints studied. Fig. 2 illustrates each companies' % total degradation target as an endpoint for drugs to be considered "reactive". Figs. 3 and 4 show the amount of chemical stress each company imparts on a drug to ensure all pharmaceutically-relevant degradation pathways have been identified.

The collation of stress conditions summarized in Figs. 2-4 plots are considered by contributing companies as representative of a comprehensive forced degradation study plan. As reported in the Campbell et al. (2022) benchmarking study, solid DPs need not be subjected to solution phase stress in well-designed forced degradation studies that include DS stress (solution and solid phase) and DP stress (solid phase).[1]

Nine of the ten participating pharmaceutical companies contributing forced degradation best practice survey data captured in Figs. 2-4 also contributed data to the Campbell et al. (2022) benchmarking study which reported; "In total, 173 degradation products were observed in accelerated and/or long-term stability studies for the 62 DPs, and these degradation products were sufficiently accounted for by forced degradation studies that included only DS stressing (in solution and in the solid state) and DP stressing (in the solid state)."[1] Campbell et al. continues "Interestingly, despite some differences in forced degradation practices that may exist amongst the pharmaceutical companies participating in this benchmarking study, the cross-industry evaluation presented in this paper demonstrates that current best practices within the industry are suitable for discovering degradation pathways relevant to the long-term stability

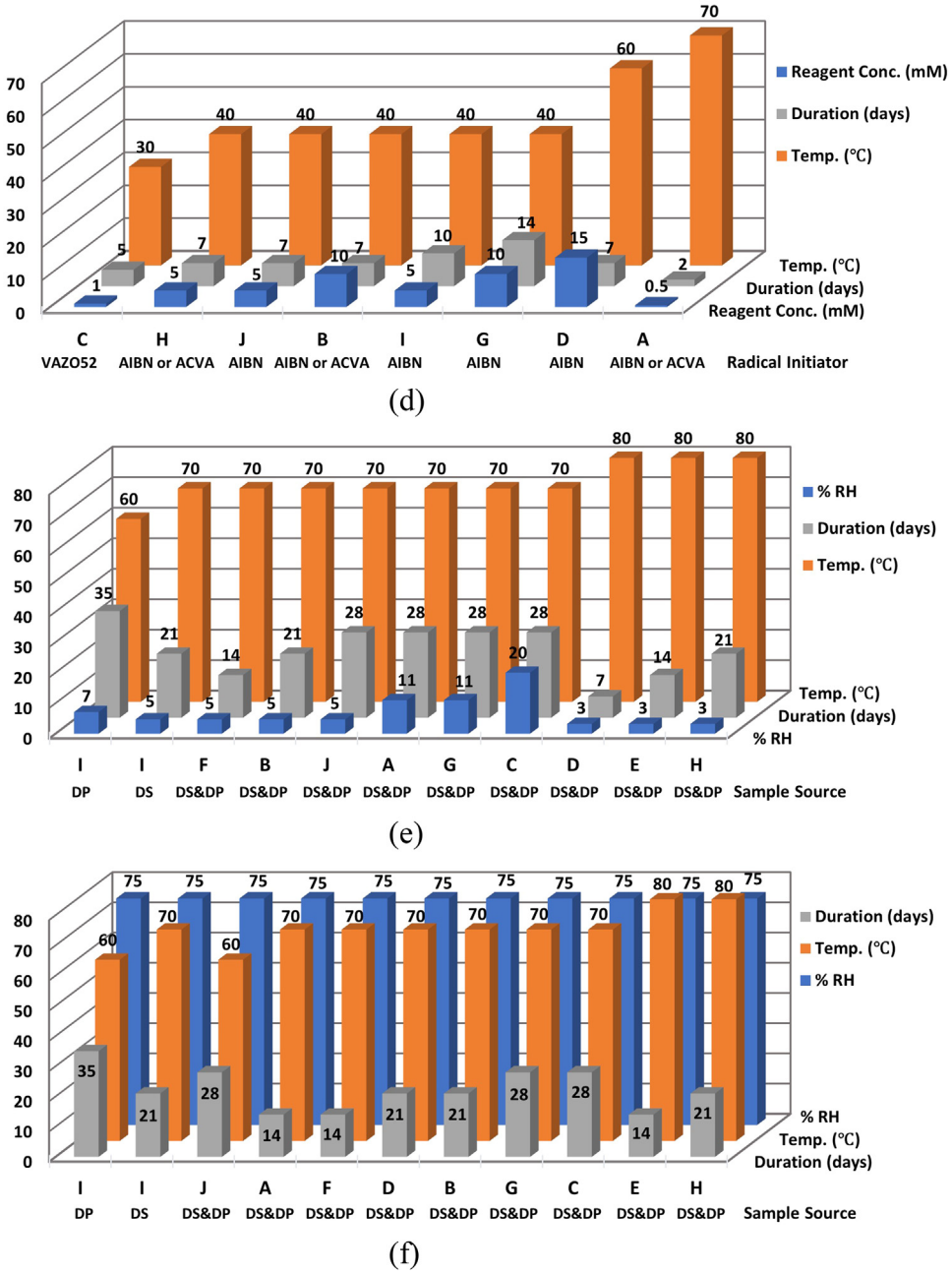

Figure 3. Continued.

of solid dosage form drug products."[1] Considering the significant company overlap in Figs. 2-4 survey data and the Campbell et al. (2022) benchmarking study data sets, the forced degradation endpoints provided by the companies are proposed to be scientifically justified, hereafter referred to as "verified" endpoints.

Endpoint 1 - % Total Degradation

The endpoint data for solution phase (i.e., DS hydrolysis and oxidation) and solid phase (i.e., DS and solid DP thermal and light) stress provided by the ten companies are generally well aligned. A verified endpoint range of 5-25% total degradation for a "reactive" drug is presented in Fig. 2.

Fig. 2(a) plots individual company's DS acid and base hydrolysis solution phase stress, ranging from 5-25% total degradation. At the low end of the range, six companies target 5% total degradation, and four companies target 10%. At the high end of the range, three companies target 15% total degradation, five companies target 20% and two companies target 25%.

Fig. 2(b) plots individual company's DS oxidative (e.g., transition metal, peroxide and/or radical initiator) solution phase stress, ranging from 0-25% total degradation. Eight of the ten companies have identical oxidative and hydrolysis stress % total degradation target ranges except for company F and company J.

Fig. 2(c) plots individual company's solid phase DS and DP % total degradation range. Fig. 2(c) outcomes are generally aligned with solution stress endpoints (e.g., 5-25% total degradation) and the construct

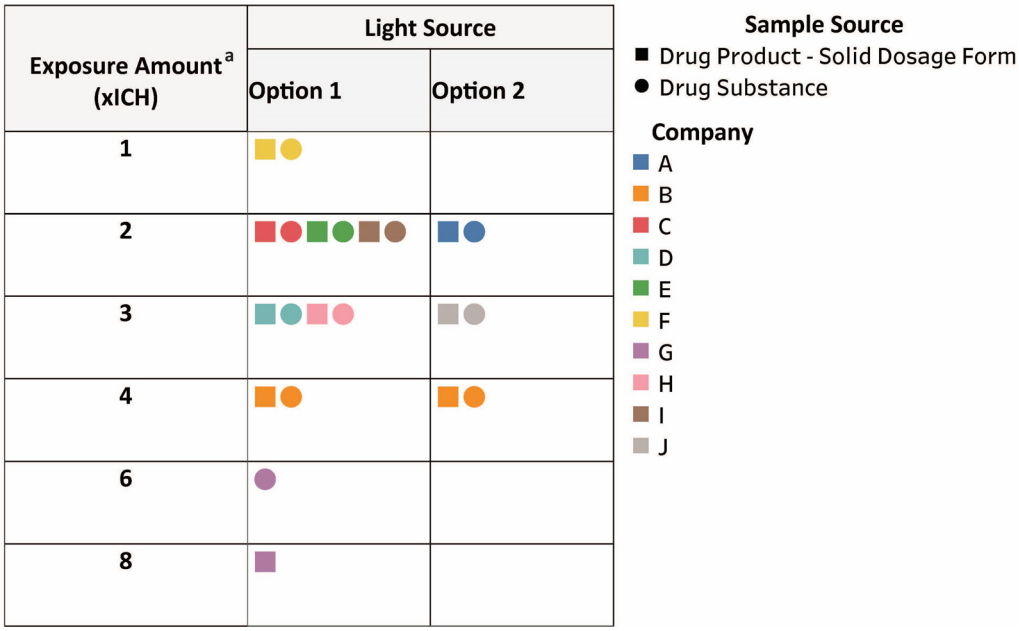

Figure 4. DS and DP photostress ten global pharmaceutical companies impart on a drug to ensure all pharmaceutically-relevant degradation pathways have been identified. [a] Photoexposure amounts relative to ICH Q1B confirmatory recommendations (i.e., 1X ICH, 200 W-h/m2 in the UV range (320-400 nm) and 1.2 million lux-h in the visible range (400-800 nm)). Option 1 light source reported values represent the visible region of light; Option 1 light source (i.e., xenon or metal halide) overexpose in the UV region of light at 2-3X ICH that of the visible region exposure amount with dependencies on filters used.

of a "reactive" drug; however, four companies target between 0% and ≤ 25% total degradation. The variability at the low end of the range can be explained by the typical stability result observed for solid drug substances and drug products; less degradation is generally observed in solid phase stress compared to solution phase stress. When companies perform solid phase stress and apply an amount of chemical stress to ensure all pharmaceutically-relevant degradation pathways have been identified (Figs. 3 and 4), it has been the authors' experience that rarely is 5% or more total degradation observed; which in turn creates the opportunity for the potential outcomes described in Table 11.

Range of conditions studied (Fig. 2(a)-(c)): 5-25% total degradation

Recommended Endpoint 1: 5-20% total degradation for a "reactive" drug

Practical Application of Endpoint 1: 5-20% Total Degradation

The practical application of reaching endpoint 1 (degradation in the range of 5-20%), as described in Table 2, is the resulting degradation profile should reflect the most relevant potential degradation pathways available to the drug under the specific stress condition. There are two possible outcomes for reaching endpoint 1. Examples scenarios provided below; see Endpoint 2 Sections below for RAPRS for individual stress conditions.

(1) 5-20% Total Degradation (RAPRS not reached)

A DS solution phase acid stress condition exposed to 0.01N HCl at ambient temperature for 24 hours reaching 12% total degradation (i.e., DS assay loss) falls short of the amount of chemical stress represented by RAPRS Endpoint 2 but satisfies the criteria for Endpoint 1.

(2) 5-20% Total Degradation (RAPRS reached)

A DS solution phase acid stress condition exposed to 0.1N HCl at 60 °C for 7 days reaching 12% total degradation would satisfy the criteria for reaching Endpoint 1 and Endpoint 2 (RAPRS, see Section entitled "Endpoint 2 - Hydrolytic Stress") concurrently.

In both instances the stress exposure would be considered complete, the potential degradation profile would be determined, and both mass balance and peak purity would be assessed.

Endpoint 2 - "Amount of Stress" for Individual Stress Conditions

The amount of chemical stress imparted on a drug for an individual stress conditions is dependent on the experimental condition

Table 2
Forced degradation endpoint 1 and actions.

Endpoint 1: % Total Degradation		Actions for Endpoint 1
% Total Degradation	Reactivity Descriptor	
5-20%	**Reactive** (RAPRS Endpoint 2 not reached)	Determine potential degradation profile.[a] Assess mass balance and peak purity.[b]
5-20%	**Reactive** (RAPRS Endpoint 2 reached concurrently)	Determine potential degradation profile.[a] Assess mass balance and peak purity.[b]

[a] The potential degradation profile is the set of degradation products observed during forced degradation that have the potential to form in accelerated and long-term stability.
[b] Peak purity analysis has verified the purity of the peak representing the active component(s).

Table 3
Number days of thermal stress calculated to reach 6 and 8.4 months at 40°C providing Arrhenius projected kinetic equivalence of NLT 2.5 years at 25°C and NLT 2.0 years at 30°C long-term storage, respectively.

Thermal Stress				Arrhenius Projected Kinetic Equivalent[a]					
	Duration (days)			Accelerated Stability	Long-Term Stability				
Temp. (°C)	(E_a = 19.87 kcal/mol)[19]	(E_a = 27.3 kcal/mol)[64]	(E_a = 29.8 kcal/mol)[2]	Number of months at 40 °C	Number of years at 30 °C			Number of years at 25 °C		
50	68	47	42	6.0	(E_a = 19.87 kcal/mol)	(E_a = 27.3 kcal/mol)	(E_a = 29.8 kcal/mol)	(E_a = 19.87 kcal/mol)	(E_a = 27.3 kcal/mol)	(E_a = 29.8 kcal/mol)
60	26.9	13.2	10.5							
70	11.2	4.0	2.8							
80	4.9	1.3	0.8		1.4	2.1	2.5	2.5	4.6	5.6
50	95	66	58	8.4	(Ea = 19.87 kcal/mol)	(Ea = 27.3 kcal/mol)	(Ea = 29.8 kcal/mol)	(Ea = 19.87 kcal/mol)	(Ea = 27.3 kcal/mol)	(Ea = 29.8 kcal/mol)
60	37.5	18.4	14.4							
70	15.6	5.5	3.9							
80	6.9	1.78	1.13		2.0	3.0	3.4	3.5	6.4	7.8

[a]Note: the effects of %RH are deliberately not included in the calculations (see text for more detail)

inputs (e.g., reagent choice, reagent concentration, temperature, duration, etc.). The experimental inputs for individual companies are shown in Figs. 3 and 4 and discussed in sections below.

Endpoint 2 - Thermal/Humidity Stress

The experimental conditions reported in Fig. 3(e) and (f) for DS and DP solid phase stress are temperature, % relative humidity (%RH) and duration. All ten companies perform thermal stress at low and high humidity. At thermal (low humidity) stress, seven of ten companies achieved desired %RH (20% or lower) by staging the drug in ovens where humidity is uncontrolled and estimated to be <10 %RH. At thermal (high humidity) stress, all ten companies are aligned and stressing at 75% RH (Fig. 3(f)). It is worth noting that considerations for maximum temperatures and %RH may need to be given for DS or solid dosage form DP that undergo significant phase changes (e.g., melting or deliquescence).

For solid phase DS and DP thermal stress, as discussed in the Introduction Section entitled "Thermal/Humidity Stress", the temperature and duration should achieve a minimum duration of greater than the kinetic equivalent of the last time point in an accelerated stability study (e.g., 6 months at 40 °C for room temperature storage), with due consideration given to the final long-term storage condition and the desired shelf life. Assuming an activation energy (E_a) of 19.87 kcal/mol (83.14 kJ/mol) recommended by United States Pharmacopeia guidance, USP <1079.2>,[19] and using Table 3 from Kleinman et al.,[18] it is straightforward to calculate the duration needed for specific stress temperatures to achieve a "kinetic equivalent" to 6 months storage at 40 °C for all the data in Fig. 3(e) and (f). These calculations are illustrated in Table 3, where the durations needed for 50, 60, 70, and 80 °C to achieve this kinetic equivalent are shown (68, 26.9, 11.2, and 4.9 days, respectively). Importantly, the practice of all ten companies surveyed exceeded these durations. The same principles (i.e., projected kinetic equivalence) apply to cold storage products (e.g., API and DP).

It is noteworthy that these Arrhenius projections do not take into account the effects of humidity on the degradation rate; instead these projections focus only on the thermal effects on rates of degradation. Qualitative assessments of the effects of humidity on degradation rates and profile can be made by comparisons of the results from low humidity to the high humidity stress conditions.

It is important to consider the relevance of the USP-recommended E_a 19.87 kcal/mol to solid-state studies; this USP-recommendation is based on solution degradation studies by Kennon in 1964.[16] More recently, MacFaul et al. studied the kinetics of solution degradation of 166 drug-like compounds and found the average E_a to be 23.6 kcal/mol (98.6 kJ/mol).[22] Experimentally derived E_a values for solid-state drug degradation studies using the accelerated stability assessment program (ASAP) approach have been reported to be significantly higher than the USP-recommended 19.87 kcal/mol. In 2010 "data from solid-state degradation studies of more than 50 compounds in 100 studies at Pfizer indicated an average E_a of 29.8 kcal/mol (124.7 kJ/mol); similar results have also been obtained in a more limited data set (nine compounds in 20 studies) at Lilly".[2] More recently, a 2018 a study evaluating the average E_a for 138 solid dosage form drug products was experimentally determined to be 27.3 kcal/mol (114.2 kJ/mol).[64] Thus, it becomes clear that an assumption of 19.87 kcal/mol for the E_a is a conservative assumption, especially for solid-state degradation.

Table 3 illustrates the impact an assumed E_a value can have on kinetic equivalence calculations. For example, to achieve a thermal kinetic equivalent to 40 °C for 6 months, stressing at 60 °C decreases from 26.9 days to 13.2 days to 10.5 days when the projections use an E_a of 19.87 kcal/mol, 27.3 kcal/mol, and 29.8 kcal/mol, respectively. Arrhenius kinetic equivalence calculations were also made for the least and most energetic thermal stress conditions shown in Fig. 3(e) and (f), projected to durations at 40 °C, 30 °C, and 25 °C; these projections are shown in Table 4. Notably, all these projections exceed 6 months at 40 °C. Arrhenius projections to the long-term storage conditions of 25 °C (Zone II)[17] all exceed 2 years, with a range of 2.6 to 145.2 years

Table 4
Calculated Arrhenius projected kinetic equivalent duration at 40 °C, 30 °C, and 25 °C for the least and most stress thermal conditions reported in company survey data set in; Fig. 3 (e) and (f).

Amount of Thermal Stress	Temp. (°C)	Duration (days)	E_a [19,64,2] (kcal/mol)	Number of months at 40 °C	Number of years at 30 °C	Number of years at 25 °C
Least	60	28	19.87	6.3	1.5	2.6
			27.3	12.8	4.5	9.7
			29.8	16.8	6.6	15.1
Most	80	21	19.87	25.7	6.1	10.7
			27.3	33.1	11.7	25.1
			29.8	156.5	63.3	145.2

for the least and most stressful conditions. It should also be acknowledged that the Arrhenius projection for the least stressful condition to 30 °C is less than 2 years (i.e., 1.5 yr.). This is an important consideration when the long-term storage condition is 30 °C (Zones III, IVa, IVb).[17] In order to address the concern that targeting a kinetic equivalent of 40 °C for 6 months may not be sufficient for Zone IVb storage conditions, we performed additional Arrhenius calculations, where the goal was to ensure thermal stress conditions, with an assumed E_a of 19.87 kcal/mol, would be kinetically equivalent to at least 2 years at 30 °C. These calculations revealed that for 30 °C long-term storage, the stress conditions should target a kinetic equivalent of not less than (NLT) 8.4 months at 40 °C as shown in Table 3.

Recommended endpoints for thermal stress are summarized in Tables 5 and 6. In addition, we recommend:

- High humidity: 75% RH
- Low humidity: 0-20% RH
- High and low humidity temperatures and durations be the same (to establish if a sensitivity to humidity exists)
- Upper temperature limit of 80 °C

Endpoint 2 - Hydrolytic Stress

All ten companies report hydrochloric acid and sodium hydroxide as the most widely used reagents for acid and base hydrolysis for DS stress testing (Fig. 3(a)). Two strengths of acid and base are reported, 0.1 N and 1 N, HCl for low pH and NaOH for high pH conditions. The conditions used by various companies are summarized Table 7 along with the RAPRS Endpoint 2. The recommended upper temperature limit is 80 °C for acid and base stress. Alternative temperatures and durations, NLT the Arrhenius projected kinetic equivalent to RAPRS (assuming E_a equal to 19.87 kcal/mol), are acceptable.

Endpoint 2 - Peroxide Stress

All ten companies evaluate peroxide mediated oxidation with nine of the ten stressing the DS with hydrogen peroxide as the reagent of choice. Two strengths of hydrogen peroxide solutions were used, 0.3% and 3% (w/w), and the temperatures ranged from 25-40 °C, consistent with recommendations by Boccardi and Baertschi et al. discussed in the Introduction Section entitled "Oxidative Stress: Peroxide". While the use of co-solvents was not defined in this data set, the two most commonly used co-solvents are methanol and acetonitrile; further discussion can be found elsewhere.[2] Data from company E is not presented in Fig. 3(c). Company E utilizes N-methylpyrrolidone (NMP) as an alternative oxidant and cosolvent to probe peroxide and radical-mediated oxidative degradation pathways.[34] The conditions used by various companies are summarized in the Table 8 along with the RAPRS Endpoint 2. The recommended upper temperature limit is 40 °C for hydrogen peroxide stress.[2,23] We recommend avoiding or minimizing the use of acetonitrile as a cosolvent as reaction with hydrogen peroxide can form peroxycarboximidic acid, an oxidizing species which is more reactive than hydrogen peroxide and can undergo reaction with weaker nucleophiles.[31] When interpreting the results of the peroxide stress, the pH should

Table 5
Recommended thermal stress (low and high humidity); Endpoint 2 (RAPRS) for a 25 °C storage product; calculated kinetic equivalence to 6 months at 40 °C accelerated and 2.5 years at 25 °C assuming Arrhenius kinetics.

RAPRS		E_a [19] (kcal/mol)	Number of months at 40 °C	Number of years at 25 °C
Temperature	Duration			
50 °C	68 days	19.87	6	2.5
60 °C	27 days	19.87	6	2.5
70 °C	11 days	19.87	6	2.5
80 °C	5 days	19.87	6	2.5

Table 6
Recommended thermal stress (low and high humidity); Endpoint 2 (RAPRS) for 30 °C storage product; calculated kinetic equivalence to 8.4 months at 40 °C accelerated and 2.0 years at 30 °C assuming Arrhenius kinetics.

RAPRS		E_a [19] (kcal/mol)	Number of months at 40 °C	Number of years at 30 °C
Temperature	Duration			
50 °C	95 days	19.87	8.4	2.0
60 °C	38 days	19.87	8.4	2.0
70 °C	16 days	19.87	8.4	2.0
80 °C	7 days	19.87	8.4	2.0

Table 7
Range of hydrolytic conditions studied and recommended amount of pharmaceutically-relevant stress (RAPRS).

	Conditions	Hydrolytic			
	Reagent	HCl or NaOHa			
Range of Conditions Studied (Fig. 3a)	Reagent Concentration-Normality (N)	0.1	0.1	1	1
	Temperature (°C)	60	80	50	70
	Duration-days	7	14	7	14
RAPRS	Reagent Concentration-Normality (N)	0.1		1	
	Temperature (°C)	60		50	
	Duration-days	7		7	

[a]Other strong acids or bases can be used.

Table 8
Range of peroxide oxidative conditions studied and recommended amount of pharmaceutically-relevant stress (RAPRS).

	Condition	Peroxide Oxidation[a]			
	Reagent	hydrogen peroxide			
Range of Conditions Studied (Fig. 3c)	Reagent Concentration % (w/w)	0.3	0.3	3	3
	Temperature (°C)	25	25	30	40
	Duration-days	3	5	2	7
RAPRS[b]	Reagent Concentration % (w/w)	0.3		3	
	Temperature (°C)	Ambient thru 40 °C		Ambient thru 40 °C	
	Duration-days	3		2	

[a] Other oxidative stress conditions are discussed in the Introduction Section entitled "Other Oxidative Stress Conditions".
[b] NMP: water, (1:1 v/v, 10:1 air/liquid ratio, 2 weeks at 80°C) are alternative reagents/conditions to probe peroxide mediated degradation pathways.

be considered for DS containing amines since protonation of the nitrogen to the cation form reduces their oxidation rate.

Endpoint 2 - Radical-Mediated Stress

Nine of the ten companies evaluate radical-mediated autoxidation using azoalkane radical initiators with AIBN or ACVA being the most common reagents of choice. Data from companies E and F are not presented in Fig. 3(d); Company E utilizes NMP as an alternative oxidant and cosolvent to probe peroxide and radical-mediated autoxidation degradation pathways in a single stress condition.[34] Radical-mediated stress is part of company F early phase forced degradation practices; however, it is not included in late phase forced degradation studies due azoalkane sourcing challenges. The conditions used by various companies are summarized in the Table 9 along with the RAPRS Endpoint 2. The recommended upper temperature limit is 40 °C to avoid non-azoalkane radical initiator catalyzed reactions (e.g. hydrolysis). A 3-10% (v/v) addition of methanol is recommended to quench more reactive oxygen species (e.g., alkoxy radicals).[31]

While the diversity of radical initiator reagents, concentrations, temperatures, and durations varies significantly across the 10 companies, the overall goal should be to produce roughly the amount of peroxy radicals formed by AIBN at ~5 mM for 2 days at 40 °C.[23] Boccardi calculated the equivalency to VAZO52 at ~5 mM for 2 days to be 25 °C. The equivalency for other azo radical initiators can be estimated using the published 10 hour half-lives:[31]

- AIBN (2,2′-Azobisisobutyronitrile), 65 °C
- ACVA (4,4′-Azobis(4-cyanovaleric acid)), 69 °C
- AAPH (2,2′-Azobis (2-methylpropionamidine)dihydrochloride)), 56 °C
- VAZO52 (2,2′-Azobis(2,4-dimethylvaleronitrile)), 51 °C

As discussed in the Introduction Section entitled "Oxidative Stress: Radical Initiators", VAZO52 can be used at 25 °C to produce a similar level of peroxy radicals over 2 days; company C uses VAZO52 at just 0.3 mM, but the temperature is 30 °C and the duration is 5 days, providing an acceptable level of peroxy radicals over the duration of the test. The practices of the other 7 companies that use azoalkane initiators also provide an acceptable level of peroxy radicals over the duration, although most exceed the recommendations of Harmon and Boccardi.[31]

Endpoint 2 - Transition Metal Stress

Nine of the ten companies stress the DS with transition metals and report using Fe^{3+} and/or Cu^{2+} salts to probe SET mediated oxidative pathway (Fig. 3(b)). The metal concentrations vary significantly (from 0.1-20 mM), as do the duration (1-14 days) and temperature (25-50 °C). The conditions used by various companies are summarized in Table 10 along with the RAPRS Endpoint 2. The recommended upper temperature limit is 40 °C to avoid non-metal catalyzed reactions (e.g., hydrolysis). Higher metal concentrations increases risk of metal salt precipitation and acidification of test solution.

While it may be difficult to deduce consensus conditions and endpoint from these diverse conditions, a review of the Introduction Section entitled "Oxidative Stress: Transitions Metals", and especially discussions by Boccardi,[23] Harmon,[31] and Baertschi et al.,[2] along with the ANVISA recommendations,[38] we conclude that transition metal concentration can be quite low and still be effective (e.g., 0.1 mM), the temperature in the range of 25-40 °C is not critical, and relevant reactions are relatively fast (i.e., < or = 1 day). To further justify low metal concentration we can calculate a non-catalyzed SET (i.e., worse case) reaction for a DS based on the following assumptions: DS sample concentration of 0.4 mg/mL, DS formula weight equal to 400 g/mol, and Fe^{3+} or Cu^{2+} salt

Table 9
Range of free radical oxidative conditions studied and recommended amount of pharmaceutically-relevant stress (RAPRS).

	Conditions	Free Radical Oxidation[a,b]										
	Reagent	AIBN						ACVA			VAZO52	AAPH
Range of Conditions Studied (Fig. 3d)	Reagent Concentration (mM)	0.5	5	5	10	10	15	0.5	5	10	0.3	NA
	Temperature (°C)	70	40	40	40	40	60	70	40	40	30	NA
	Duration-days	2	7	10	7	14	7	2	7	7	5	NA
RAPRS[c,d]	Reagent Concentration (mM)	5						5			5	5
	Temperature (°C)	40						40			25	30
	Duration-days	2						2			2	2

NA = Not studied.
[a] Other oxidative stress conditions are discussed in the Introduction Section entitled "Other Oxidative Stress Conditions".
[b] A 3-10% (v/v) addition of methanol is recommended.
[c] NMP: water, (1:1 v/v, 10:1 air/liquid ratio, 2 weeks at 80°C) are alternative reagents/conditions to probe radical-mediated degradation pathways.
[d] Alternative radical initiators providing similar levels of peroxy radicals as the AIBN system are acceptable.

Table 10
Range of metal oxidation conditions studied and recommended amount of pharmaceutically-relevant stress (RAPRS).

	Condition	Metal Oxidation[a]						
	Reagent	Fe^{3+} and/or Cu^{2+} salts						
Range of Conditions Studied (Fig. 3b)	Reagent Concentration (mM)	0.1	0.3	1	5	10	10	20
	Temperature (°C)	50	25	25	40	25	25	25
	Duration-days	3	14	5	10	7	10	1
RAPRS	Reagent Concentration (mM)	0.1 - 1						
	Temperature (°C)	25 - 40						
	Duration-days	1						

[a] Other oxidative stress conditions are discussed in the Introduction Section entitled "Other Oxidative Stress Conditions".

concentration of 0.1 mM. In this instance, a quantitative SET reaction with the DS would correspond to a 10% loss of the DS.

Endpoint 2 - Photostress

All ten companies perform photostress (Fig. 4). As described in ICH Q1B such studies "may involve the DS alone and/or in simple solutions/suspensions to validate the analytical procedures".[47] The experimental variables reported in Fig. 4 for DS and DP solid phase stress include light source (e.g., Option 1 or 2) and photoexposure in excess to ICH Q1B confirmatory recommendations (200 W-h/m2 in the UV range (320-400 nm) and 1.2 million lux-h in the visible range (400-800 nm)). Light source choice and the degree of excess of photoexposure relative to confirmatory recommendations defines the severity of stress imparted on the drug. The majority of companies are using Option 1 light source and exposing at 2-3X ICH confirmatory recommendation, consistent with recommendations in the literature.[45,50,52]

RAPRS:

- Solid state: 2-3 x ICH Q1B minimum confirmatory exposure in both UV and visible, using either Option 1 or 2 light sources, with sample presentation in alignment with ICH Q1B

Practical Application of Endpoint 2: RAPRS

The practical application of reaching the RAPRS Endpoint 2 while executing a forced degradation condition suggests four possible outcomes, each with a unique designation as described in Table 11. Examples scenarios provided below; see Endpoint 2 Sections above for RAPRS for individual stress conditions.

(1) RAPRS ("Stable", 0% Total Degradation)

To illustrate a 0% total degradation outcome example (e.g., no significant change in assay): a solid DS exposed to 70 °C/75% RH for 21 days results in 0.3% total degradation (i.e., 0.3% loss of assay) with no degradation products observed above reporting threshold. The 0.3% loss of assay is considered within the analytical variability of the method and therefore would satisfy the criteria for reaching RAPRS Endpoint 2; however peak purity should be assessed.

(2) RAPRS ("Moderately Stable", > 0% to < 5% Total Degradation)

A solid DS exposed to 60° C/75% RH for 38 days resulting in 2% total degradation (i.e., 2% loss of assay) with the potential degradation profile presenting three degradation products would satisfy the criteria for reaching RAPRS Endpoint 2. The three degradation products would be included in the optimization and validation of the SIM (see Fig. 1). Additionally, mass balance and peak purity would be assessed. The drug would be considered "moderately stable" to the stress condition. In a similar stress scenario, a DS solid phase thermal (high humidity) stress condition exposed to 60° C/75% RH for 38 days results in 4% total degradation (relative to initial) with no reportable degradation products. This scenario would also satisfy the criteria for reaching RAPRS Endpoint 2; however peak purity should be assessed and a mass balance investigation may be warranted.[63]

(3) RAPRS ("Reactive", 5-20% Total Degradation)

A DS exposed to 3% (w/w) hydrogen peroxide in solution at ambient temperature for 2 days reaches 10% total degradation (i.e., 10% loss of assay), satisfies the criteria for reaching both Endpoints 1 and 2 concurrently. The potential degradation profile includes three degradation products that would be included in the optimization and validation of the SIM (see Fig. 1). Additionally, mass balance and peak purity would be assessed. The drug would then be considered "reactive" to the stress condition, with due consideration of pH for the stress condition which can impact the rate of oxidation.[31]

(4) RAPRS (Over degraded, ≥20% total degradation)

A DS exposed to 0.1 N aqueous NaOH at 60° C for 7 days exhibited 50% total degradation. In this hypothetical example, a sample was stressed for 7 days with a single kinetic time point analyzed; further actions might include repeating the experiment and imparting less stress than the RAPRS for the base condition (e.g.,

Table 11
Forced degradation Endpoint 2 and actions.

Endpoint 2: RAPRS		Actions for Endpoint 2
% Total Degradation	Reactivity Descriptor	
0%[a]	**Stable**	Assess peak purity[b]
> 0% to < 5%	**Moderately Stable**	Determine potential degradation profile (when applicable). Assess mass balance and peak purity.
5-20%	**Reactive** (Endpoint 1 reached concurrently)	Determine potential degradation profile. Assess mass balance and peak purity.
>20%	**Over Degraded**	Consider repeating the stress condition and imparting less than RAPRS until 5-20% total degradation is reached.

[a] 0% total degradation +/- the analytical variability of the method. A potential degradation profile is not observed.
[b] Peak purity analysis has verified the purity of the peak representing the main (parent) analyte.

Table 12
Summary of recommended forced degradation endpoints.

	Endpoint 1: % Total Degradation	Endpoint 2: RAPRS (≤ 20% total degradation)									
Solution Phase Stress[a] DS	5-20%	**Degradation Pathway**	**Hydrolysis**		**Oxidation**						
		Study Type	**Acid/Base**		**Metals**	**Peroxide**[b]		**Radical Initiator**[b,c]		
		Reagent	HCl/NaOH		Fe^{3+} and/or Cu^{2+} salts	H_2O_2		AIBN, ACVA	AAPH	VAZ052
		Reagent Concentration	0.1 N	1.0 N	0.1-1 mM	0.3% (w/w)	3% (w/w)	5 mM	5 mM	5 mM
		Temperature	60 °C	50 °C	25-40 °C	25-40 °C	25-40 °C	40 °C	30 °C	25 °C
		Duration	7 d	7 d	1 d	3 d	2 d	2 d	2 d	2 d
Solid Phase Stress DS & DP	5-20%	**Degradation Pathway**	**Thermal**[f]							**Photostability**
		Study Type	Low Humidity				High Humidity			Option 1 or 2
		Temperature	50 °C	60 °C	70 °C	80 °C	50 °C	60 °C	70 °C	80 °C	NA
		Humidity	0-20% RH				75% RH				NA
		Duration/Exposure For 25 °C long-term storage[d]	68 d	27 d	11 d	5 d	68 d	27 d	11 d	5 d	2-3 X ICH
		Duration/Exposure For 30 °C long-term storage[e]	95 d	38 d	16 d	7 d	95 d	38 d	16 d	7 d	

[a] For all of the solution stress conditions, the presumed concentration of DS is 0.1-1 mg/mL.
[b] NMP: water, (1:1 v/v, 10:1 air/liquid ratio, 2 weeks at 80 °C) are alternative reagents/conditions to probe radical-mediated and peroxide oxidative degradation pathways.
[c] 3-10% (v/v) addition of methanol is recommended to quench more reactive oxygen species (e.g., alkoxy radicals).
[d] Provides Arrhenius projected kinetic equivalence of NLT 6 months at 40 °C and 2.5 year storage at 25 °C assuming conservative E_a of 19.87 kcal/mol.
[e] Provides Arrhenius projected kinetic equivalence of NLT 8.4 months at 40 °C and 2.0 year storage at 30 °C assuming conservative E_a of 19.87 kcal/mol.
[f] Arrhenius derived durations (Section entitled "Endpoint 2 - Thermal/Humidity Stress") do not take into account the effects of humidity on the degradation rate. Comparison of low humidity to high humidity results allows a qualitative assessment to determine if a sensitivity to humidity exists.

performing time points < 7 days, decreasing the temperature and/or decreasing the reagent concentration) until 5-20% total degradation is reached. A >20% total degradation result can be considered on a case-by-case basis.

Application of the proposed two-endpoint classification construct helps to provide a clearer path to determine if a drug can be considered "stable" to a particular forced degradation stress condition. To deem a drug "stable" to a condition the researcher would apply the RAPRS Endpoint 2 and conclude 1) drug assay loss is 0% +/- the analytical variability of the method, 2) a potential degradation profile is not observed, 3) mass balance assessment is deemed acceptable and 4) main band peak purity assessment has verified the purity of the main drug analyte peak. In rare circumstances, additional stress may need to be applied when a drug is deemed stable to all conditions by application of RAPRS since degradation products are required to facilitate the development of stability-indicating methods.[2]

Endpoint 2 Considerations – What is Considered "Too Severe"?

An amount of stress that significantly exceeds the conditions depicted in Figs. 2-4, or its "kinetic equivalent", has not been verified in this study and is therefore not recommended. Harsher conditions and endpoints can increase the risk of creating non-relevant degradation. For example, Fig. 3(a) acid stress verified ranges are temperature (50-80 °C), reagent concentration (0.1 or 1 N) and duration (5-21 days). A combination of maximum reagent concentration (1 N HCl), maximum temperature (80 °C), and maximum duration (21 days) for acid stress exceeds the most stress reported (i.e., 1 N HCl at 80 °C for 14 days), is therefore not a verified endpoint and not recommended. In the case of thermal stress (Fig. 3 (e) and (f)), the verified ranges for temperature are 60-80 °C, 0-20% RH for low humidity and 75 %RH for high humidity. While humidities higher than 75% can be used for the humidity stress, the risk of physical changes (e.g., deliquescence) should be considered, especially at higher temperatures and in the presence of excipients (e.g., DP). Temperatures above 80 °C are not recommended because of the risk of triggering non-relevant degradation pathways.[21] While the reported duration varies from 14-35 days, it should correspond to a kinetic equivalent of not less than 40 °C for 6 months for 25 °C long-term storage and 8.4 months for 30 °C long-term storage (assuming E_a equal to 19.87 kcal/mol), as described above in Section entitled "Endpoint 2 - Thermal/Humidity Stress". It is noteworthy that we are not recommending a maximum duration for the thermal (low and high humidity) stress condition, since there may be cases where longer timeframes are needed in order to project to the desired shelf life. For example, if the Ea is known to be significantly below 19.87 kcal/mol, or if the desired long-term shelf life is significantly longer than 3 years.

Conclusions

This article provides readers with an industry perspective and practical recommendations for preparing a well-designed forced degradation study plan that includes forced degradation stress conditions and scientifically justified endpoints for small molecule DS and solid dosage form DP; summarized in Table 12. Due to the lack of agreement in the scientific literature and global regulatory guidances regarding what constitutes appropriate endpoints for pharmaceutical forced degradation studies, we have introduced a two-endpoint classification designation. Stress endpoints can be either the amount of degradation in the range of 5-20% (Endpoint 1) or the amount of stress needed to ensure all pharmaceutically-relevant degradation

pathways have been identified (Endpoint 2), whichever is reached first, without exceeding 20% total degradation. Endpoint 2 would be applied to demonstrate a drug is "stable" to the specific stress condition. The authors have provided specific endpoint recommendations for each stress condition (Table 12). These recommendations were derived from an extensive review of the literature, regulatory guidances, and a forced degradation data set from ten global pharmaceutical companies. We assert that application of these recommendations will help the industry to plan and execute well-designed forced degradation studies that include appropriate science based endpoints. It is the entirety of the forced degradation study, and not a single stress condition result, that allows for the comprehensive pharmaceutically-relevant degradation profile of a drug to be determined.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

1. Campbell JM, Foti C, Wang C, et al. Assessing the relevance of solution phase stress testing of solid dosage form drug products: a cross-industry benchmarking study. *J Pharm Sci*. 2022;111(2):298–305.
2. Baertschi SW, Jansen PJ, Alsante KM. Chapter 2/stress testing: a predictive tool. In: Baertschi SW, Alsante KM, Reed RA, eds. *Pharmaceutical Stress Testing: Predicting Drug Degradation, Second Edition*. 2nd ed CRC Press; 2011.
3. Singh S, Junwal M, Modhe G, et al. Forced degradation studies to assess the stability of drugs and products. *TrAC, Trends Anal Chem*. 2013;49:71–88.
4. Reynolds DW, Facchine KL, Mullaney JF, Alsante KM, Hatajik TD, Motto MG. Conducting forced degradation studies. *Pharm Technol*. 2002;26(2):48–56.
5. Alsante KM, Ando A, Brown R, et al. The role of degradant profiling in active pharmaceutical ingredients and drug products. *Adv Drug Deliv Rev*. 2007;59(1):29–37.
6. Alsante KM, Baertschi SW, Coutant M, Marquez BL, Sharp TR, Zelesky TC. Degradation and impurity analysis for pharmaceutical drug candidates. *Separation Science and Technology*. Elsevier; 2011:59–169.
7. Klick S, Muijselaar PG, Waterval J, et al. Stress testing of drug substances and drug products. *Pharm Technol*. 2005;29(2):48–66.
8. Blessy M, Patel RD, Prajapati PN, Agrawal Y. Development of forced degradation and stability indicating studies of drugs—a review. *J Pharm Anal*. 2014;4(3):159–165.
9. *Pharmaceutical Stress Testing: Predicting Drug Degradation*. 1st edition Informa Healthcare; 2005.
10. *Pharmaceutical Stress Testing: Predicting Drug Degradation*. 2nd edition CRC Press; 2011.
11. Alsante KM, Martin L, Baertschi SW. A stress testing benchmarking study. *Pharm Technol*. 2003;27(2):60–73.
12. Singh S, Bakshi M. Stress test to determine inherent stability of drugs. *Pharm Technol*. 2000;4:1–14.
13. Grimm W, Thomae K. Chapter XVI. In: Krummen K, ed. *Stability Testing of Drug Products: Scientific Criteries, Guidelines and Official State Requirements in Europe, Japan, and USA*. Wissenschaftliche Verlagsgesellschaft mbH Stuttgart; 1987.
14. Florey K. *Analytical Profiles of Drug Substances*. Academic Press; 1991.
15. Baertschi SW, Reynolds DW. Chapter 1/introduction. In: Baertschi SW, ed. *Pharmaceutical Stress Testing: Predicting Drug Degradation*. 1st edition Informa Healthcare; 2005.
16. Kennon L. Use of models in determining chemical pharmaceutical stability. *J Pharm Sci*. 1964;53(7):815–818.
17. ICH. ICH guideline Q1A (R2) stability testing of new drug substances and products. *International Conference on Harmonization Expert Working Group 24*. 2003.
18. Kleinman MH, Elder D, Teasdale A, Mowery MD, McKeown AP, Baertschi SW. Strategies to address mutagenic impurities derived from degradation in drug substances and drug products. *Org Process Res Dev*. 2015;19(11):1447–1457.
19. 2021. USP <1079.2>(2021) risks and mitigation for the storage and transportation of finished drug products. ed.
20. Stewart P, Tucker I. Prediction of drug stability. Part 2. Hydroylsis. *Aus J Hosp Pharm*. 1985;15:11–16.
21. Singh R, Rehman ZU. Current trends in forced degradation study for pharmaceutical product development. *J Pharmaceut Educ Res*. 2012;3(1):54.
22. MacFaul PA, Ruston L, Wood JM. Activation energies for the decomposition of pharmaceuticals and their application to predicting hydrolytic stability in drug discovery. *MedChemComm*. 2011;2(2):140–142.
23. Boccardi G. *Oxidative Susceptibility Testing. Pharmaceutical Stress Testing*. CRC Press; 2005:227–256.
24. Ingold K. Inhibition of the autoxidation of organic substances in the liquid phase. *Chem Rev*. 1961;61(6):563–589.
25. Boccardi G, Deleuze C, Gachon M, Palmisano G, Vergnaud JP. Autoxidation of tetrazepam in tablets: prediction of degradation impurities from the oxidative behavior in solution. *J Pharm Sci*. 1992;81(2):183–185.
26. Hovorka SW, Schöneich C. Oxidative degradation of pharmaceuticals: theory, mechanisms and inhibition. *J Pharm Sci*. 2001;90(3):253–269.
27. Waterman KC, Adami RC, Alsante KM, et al. Stabilization of pharmaceuticals to oxidative degradation. *Pharm Dev Technol*. 2002;7(1):1–32.
28. Nelson ED, Harmon PA, Szymanik RC, et al. Evaluation of solution oxygenation requirements for azonitrile-based oxidative forced degradation studies of pharmaceutical compounds. *J Pharm Sci*. 2006;95(7):1527–1539.
29. Nelson ED, Thompson GM, Yao Y, Flanagan HM, Harmon PA. Solvent effects on the AIBN forced degradation of cumene: Implications for forced degradation practices. *J Pharm Sci*. 2009;98(3):959–969.
30. Watkins MA, Pitzenberger S, Harmon PA. Direct evidence of 2-cyano-2-propoxy radical activity during aibn-based oxidative stress testing in acetonitrile–water solvent systems. *J Pharm Sci*. 2013;102(5):1554–1568.
31. Harmon PA, Boccardi G. Chapter 6/oxidative susceptibility testing. In: SW B, Alsante KM, Reed RA, eds. *Pharmaceutical Stress Testing: Predicting Drug Degradation*. 2nd ed. Informa Healthcare; 2011.
32. Nefliu M, Zelesky T, Jansen P, et al. Artifacts generated during azoalkane peroxy radical oxidative stress testing of pharmaceuticals containing primary and secondary amines. *J Pharm Sci*. 2015;104(12):4287–4298.
33. Harmon PA, Kosuda K, Nelson E, Mowery M, Reed RA. A novel peroxy radical based oxidative stressing system for ranking the oxidizability of drug substances. *J Pharm Sci*. 2006;95(9):2014–2028.
34. Reynolds DW, Galvani M, Hicks SR, et al. The use of N-methylpyrrolidone as a cosolvent and oxidant in pharmaceutical stress testing. *J Pharm Sci*. 2012;101(2):761–776.
35. Schwender CF. *Levarterenol Bitartrate. Analytical Profiles of Drug Substances*. Elsevier; 1972:149–173.
36. Baertschi S. *Fundamentals of Predicting Oxidative Impurities*. Chicago, IL: AAPS Workshop on Predicting and Monitoring Impurities; 2012.
37. Nanda KK, Blincoe WD, Allain LR, Wuelfing WP, Harmon PA. Iron (III)-mediated oxidative degradation on the benzylic carbon of drug molecules in the absence of initiating peroxides. *J Pharm Sci*. 2017;106(5):1347–1354.
38. Brazilian Ministry of Health NHSAA. 2015. Establishes parameters for reporting, identification and qualification of degradation products in drug products with synthetic and semi-synthetic active substances, classified as new chemical entities, generics and branded generics, among other provisions. In ANVISA, editor RDC 53, ed.
39. Reynolds DW, Campbell JM, Johnson BS, et al. The degradation chemistry of farglitazar and elucidation of the oxidative degradation mechanisms. *J Pharm Sci*. 2017;106(4):982–993.
40. Zhu D, Zhang GG, George KLS, Zhou D. A novel accelerated oxidative stability screening method for pharmaceutical solids. *J Pharm Sci*. 2011;100(8):3529–3538.
41. Petřík J, Zůza D, Heřt J, et al. Azobisisobutyronitrile loaded on mesoporous silica particles: a new stressor for solid-state oxidative forced degradation studies. *J Pharm Biomed Anal*. 2023 115417.
42. Polizzi MA, Singhal D, Colvin J. Mechanoradical-induced degradation in a pharmaceutical blend during high-shear processing. *Pharm Dev Technol*. 2008;13(6):457–462.
43. Modhave D, Saraf I, Karn A, Paudel A. Understanding concomitant physical and chemical transformations of simvastatin during dry ball milling. *Aaps Pharmscitech*. 2020;21:1–10.
44. Iyer J, Barbosa M, Saraf I, Pinto JF, Paudel A. Mechanoactivation as a tool to assess the autoxidation propensity of amorphous drugs. *Mol Pharmaceut*. 2023.
45. Piechocki JT. *The history of pharmaceutical photostability development. Pharmaceutical Photostability and Stabilization Technology*. CRC Press; 2006:25–70.
46. Baertschi SW, Reynolds DW. Chapter 1/introduction. In: Baertschi SW, Alsante KM, Reed RA, eds. *Pharmaceutical Stress Testing: Predicting Drug Degradation, Second Edition*. 2nd ed CRC Press; 2011:28., Table 11..
47. ICH 1996. Stability testing: photostability testing of new drug substances and products. Q1B, Current Step 4.
48. Thatcher S, Mansfield R, Miller R, Davis C, Baertschi S. Pharmaceutical photostability: a technical guide and practical interpretation of the ICH guideline and its application to pharmaceutical stability–part I. *Pharm Technol*. 2001;25(3):98–110.
49. Thatcher S, Mansfield R, Miller R, Davis C, Baertschi S. Pharmaceutical photostability: a technical guide and practical interpretation of the ICH guideline and its application to pharmaceutical stability–part II. *Pharm Technol*. 2001;25(4):50–62.
50. Clapham D, Templeton AC, Klein LJ, Kleinman MH. Practical aspects of conducting photostability stress testing. In: Baertschi S, Alsante KM, Reed RA, eds. *Pharmaceutical Stress Testing: Predicting Drug Degradation*. 2nd Edition CRC Press; 2011:230–244.
51. Baertschi SW, Thatcher SR. *Sample presentation for photostability studies: problems and solutions. Pharmaceutical Photostability and Stabilization Technology*. CRC Press; 2006:201–226.
52. Baertschi SW, Alsante KM, Tønnesen HH. A critical assessment of the ICH guideline on photostability testing of new drug substances and

products (Q1B): recommendation for revision. *J Pharm Sci*. 2010;99(7): 2934–2940.
53. ICH. Validation of analytical procedures: text and methodology. *ICH Harmon Tripartite Q2 (R1)*. 2005;1(20):05.
54. ICH 2006. Impurities in new drug substances Q3A (R2). Proceedings of the international conference on harmonization of technical requirements for registration of pharmaceuticals for human use, Geneva, Switzerland 25.
55. ICH. Impurities in new drug products, Q3B (R2), current step. *ICH Harmon Tripartite*. 2006;4:1–5.
56. ICH. Assessment and control of dna reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk M7. *International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH)*. Geneva; 2014.
57. WHO. 2018. Stability testing of active pharmaceutical ingredients and finished pharmaceutical products. In preparations WHOWecosfp, editor WHO 52nd Technical Report Series, No 1010, ed.
58. (ANVISA) NHSA. 2015. Guide for obtaining degradation profile, and identification and qualification of degradation products in drug products. In ANVISA, editor Guide No 04, ed.
59. FDA 2022. Questions and answers on current good manufacturing practice requirements/laboratory controls. https://wwwfdagov/drugs/guidances-drugs/questions-and-answers-current-good-manufacturing-practice-requirements-laboratory-controls#2.
60. Kleinman MH, Baertschi SW, Alsante KM, et al. In silico prediction of pharmaceutical degradation pathways: a benchmarking study. *Mol Pharmaceut*. 2014;11 (11):4179–4188.
61. Hemingway R, Baertschi S, Benstead D, et al. In silico prediction of pharmaceutical degradation pathways: a benchmarking study using the software program Zeneth, sumitted for publication. ed.
62. Baertschi SW, Olsen B, Alsante KM, Reed RA. Chapter 5/stress testing: relation to the development timeline. In: Baertschi SW, Alsante KM, Reed RA, eds. *Pharmaceutical Stress Testing: Predicting Drug Degradation, Second Edition*. 2nd ed CRC Press; 2011.
63. Baertschi SW, Pack BW, Hyzer CSH, Nussbaum MA. Assessing mass balance in pharmaceutical drug products: new insights into an old topic. *TrAC, Trends Anal Chem*. 2013;49:126–136.
64. Waterman KC. *ASAPprime® Version 6: Algorithm-Based Data Analysis*. 2018. SOS 2018, ed., Boston, MA.