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Forced Degradation – A Review

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ABSTRACT

Forced degradation studies are used to facilitate the development of analytical methodology, to gain a better understanding of active ingredient (API) and drug product (DP) stability, and to provide information about degradation pathways and degradation products. The stability of a drug product or a drug substance is a critical parameter which may affect purity, potency and safety. Changes in drug stability can risk patient safety by formation of a toxic degradation product (s) or deliver a lower dose than expected. Therefore, it is essential to know the purity profile and behaviour of a drug substance under various environmental conditions. It is important to recognize that forced degradation studies are not designed to establish qualitative or quantitative limits for change in drug substance or drug product.

Introduction

The initial purpose of forced degradation studies is to investigate stability related properties of an API and to develop an understanding of the degradation products and pathways. These studies should also be used to evaluate the susceptibility of the drug substance to hydrolysis across a wide range of pH values. Forced degradation studies are also used to provide degraded samples for the development of stability- indicating analytical methods for the API. The information received from a forced degradation study can also be utilized in several other areas of development. Analytical methods development, formulation development and storage conditions, manufacturing –processing safety –toxicological, identification of possible genotoxic degrades identification of potential metabolites of API design/discovery. Forced degradation studies are most beneficial if done in early stage of dug development process. The reasoning for this is that these studies yield predictive information on the nature of the degrades which are valuable when assessing the appropriate synthesis routes. API salt selection and formulation development.

- To develop and validate a stability indicating method.
- To determine degradation pathways of drug substances and drug products (e, g., during development phase).
- To identify impurities related to drug substances or excipients
- To understand the drug molecule chemistry.
- To generate more stable formulations.
- To generate a degradation profile that mimic what would be observed in a formal stability study under ICH conditions
- To solve stability- related problems (e.g., Mass balance) [1].

In order to monitor possible changes to product over time, the applied analytical method (in most cases a chromatographic method) must be stability-indicating. The best case for testing the suitability of a method is using real time stability samples containing all relevant degradation products that might occur. But due to product development timelines process characteristics, excipients and other environmental factors, a forced degradation study (stress test) can serve as an alternative in a typical study, relevant stress conditions are light, heat, humidity, hydrolysis (acid\base influence)

an oxidation or even a combination of described parameters. If it is necessary to form degradation products, the strength of stress conditions can vary due to the chemical structure of the drug substance, the kind of drug product, and product specific storage requirements. An individual program must be set up in order to reach a target degradation of 5 to 20%. A higher level of degradation will be out of the scope of product stability requirements and therefore it is assumed to be unrealistic. The scope of the test is to generate degradation product in order to facilitate a method development for determination of the relevant products. Therefore, samples will be stressed in a solid form and\or in solution. Typically, the stress tests are carried out in one batch of material. For drug products the placebo should be stressed in a similar way in order to exclude those impurities that are not degradation products (e.g., impurities arising from excipients). Table 1 Illustrate typical stress conditions of API and drug product. Drugs that are poorly soluble in water can be conducted either in suspension or in solution using inert organic co solvents (e.g., DMSO, acetic acid or propionic acid). It is important to avoid co solvents that may be reactive to the drug or complicate analysis (e.g., By LC-MS). Through the use of these early development degradation studies the focus should be the garnering of as much information as possible about the chemistry of the API. To accomplish this, forced degradation studies are done on both the solid state and aqueous solution or suspension forms of the API. Furthermore, the use of analysis at multiple time points allows for approximation of rates of degradation and such testing at early time points can provide a distinction between primary and secondary degradation products. This approach allows for better degradation pathway determination.

Table 1: Typical stress conditions of API and products.

Stress condition	Examples	API		Drug Product	
		Solid	Solution/Suspension	Solid	Solution/ Suspension
Sol acid/base oxidative	0.01 TO 0.1N	-	X	-	X
	0.3% H2O2	-	X	-	X
Light	1200Luxh	X	X	X	X
Temperature	100C to 700C	X	X	X	X
Temperature/humidity	100C to 700C and 60 at 90 r.h	X	X	X	X

Note: X.recommended:

Forced degradation studies should be repeated as needed throughout the drug development process. For example, when there are no changes in API impurity profile, API salt or polymorph form. When carried out in late development such studies are referred to as confirmatory studies. Confirmatory studies are quantitative in nature. Full mass accountability of the API, its impurities and degradation products are generated from these late-stage studies. Furthermore, based upon the outcome of these studies, if necessary, new or orthogonal methods may need to be developed to account for all observed degradation. Also, confirmatory studies are typically done in phase III with one of the registration batches of the API. For drug products, confirmatory studies are done when final formulation(s) and packaging are chosen. After the confirmatory studies are completed, a report on degradation products and pathways is generated and are included in or used to support NDA fillings. The following are general conditions that should be employed when conducting forced degradation studies:

Solid State

- Heat
- Heat/humidity
- Light
- Solution and\or Suspension
- Hydrolysis at various pH s
- Buffered HCl, NaOH, Water
- Buffer solutions (used to determine if PH adjustment needed to attain maximum stability)
- Oxidative stress testing
- H2O2 (to mimic possible presence of peroxides in excipients)
- Metal ion (to mimic possible exposure during manufacture)
- Radical initiators (to mimic auto oxidation)
- Light

The general approach for carrying out the testing of samples generated from forced degradation is through the use of HPLC (LC) with either a UV or PDA detector. In most instances the initial starting method will be some generic LC method with an appropriate column to effectuate separation. Over time, this method will be refined or modified so that complete separation of the API and it de-

grades are achieved. Once a satisfactory LC method has been developed the process of identifying the structure of degrades can begin. This involves transferring the method for use with LC-MS. LC-MS is a powerful analytical tool specifically capable of proving structural elucidation. By using LC-MS, and where needed LC-NMR, information on the structure of each impurity and degradation product of an API can thus be developed. This information along with a fundamental understanding of the API's chemistry provides the means for understanding the API's degradation pathways. In addition, the elucidation and identification of these structures allows investigating whether the degradation products are known compounds that have been previously characterized or whether they are potential carcinogens or genotoxins upon identification the degradation products the results of the forced degradation can be formalized. This formalization must include both the degradation conditions used and the proposed structures of the degradation products observed for those conditions. A proposed mechanism of degradation that identifies potential degradation products and pathways can thus be understood. Armed with this, a stable formulation with proper packaging and/or storage conditions can thus be developed.

Overview of Regulatory Guidance

According to the available guidance (12-14), forced degradation studies are carried out for the following reasons:

- Development and validation of stability –indicating methodology
- Development of degradation pathways of drug substance and dug products
- Discernment of degradation products in formulations that are related to drug substances versus those that are related to non- drug substances (e.g., excipients)
- Structure elucidation of degradation products
- Determination of the intrinsic stability of a drug substance molecule.
- Are carried out in solution and/or the solid state
- Involve conditions more serve than accelerated testing (e.g., _40_c; _75% relative humidity; in excess of ICH light conditions; high and low PH, oxidation, etc.)
- Are typically carried out on one batch of material
- Include conditions that analyze thermolytic, hydrolytic, oxidative and photolytic degradation mechanisms in the drug substance and drug product (as appropriate)
- Are not parts of the formal stability program.

The FDA and International Conference on Harmonization (ICH) guidance provides very little information about strategies and principles for conducting forced degradation studies, including problems of poorly soluble drugs and exceptionally stable compounds. In particular, the issue of how much stress is adequate in stress testing is not addressed specifically. Overstressing a molecule can lead to degradation profiles that are not representative of real storage conditions and perhaps not relevant to method development. Therefore, stress –testing conditions should be realistic and not excessive. In this regard, it is the amount of stress that is important and not necessarily the extent of degradation. Indeed, some compounds may not be degrading significantly after considerable exposure to stress conditions. Also, somewhat un clear is what I should be done at each development phase from both a regulatory and a scientific perspective. Although FDA does not require degradation studies for an investigational new drug (IND) application, preliminary degradation studies are useful for the development of the stability –indicating methods that will be used during the clinical trails. This article provides a practical interpretation and summary of the available guidance and some suggestions for the best practices for conducting forced degradation studies. It represents the collective views presented by industry participants at the pharmaceutical Research and Manufacturers of America Analytical Research and Development steering committee workshop about this subject. Concise summaries, rather than lengthy quotations, of what is stated in the guidance are provided with references. Material taken from the guidance is referenced and /or explicitly stated to be expected from the guidance's [2].

Summary of Requirements at the IND Phase

Although the reporting of degradation studies is not required in IND applications, preliminary studies may be carried out to facilitate the development of stability- indicating methodology. Studies can be conducted on the drug substance and developmental formulations to test for degradation by thermolysis, hydrolysis, oxidation, and photolysis or to evaluate the potential chemical behavior of the active ingredient. A draft guidance document suggests that results of one-time stress studies should be included in phase 3 application for INDs [3].

Summary of Requirements for Marketing Application

Completed studies of the degradation of the drug substance and drug product are required at the new drug application (NDA) stage, including isolation and/or characterization f significant degradation products and full written account of the degradation studies performed [4]. Drug substance. F or degradation studies of a drug substance, FDA requests the following at the time of registration:

- Stressing the drug substance in solution or suspension at acidic and alkaline PH and under oxidation conditions
- Stressing the solid bulk drug substance at temperature and temperature-humidity conditions in excess of accelerated conditions.

- Stressing the drug substance photolytic ally in the solid state and/or in solution in excess of ICH conditions
- Demonstration of the specificity of stability- indicating methods with forced- degraded samples or with the drug substance spiked with appropriate markers
- Isolation and/or full characterization (by mans of NMR, mass spectrometry (MS), UV analysis, etc.) of all significant degradation products if possible [5]. procedures for the preparation and /or isolation (where applicable) and structure determination of the degradation products should be reported. Unsuccessful attempts to identify significant degradation products should also be documented
- The chemical and physical properties of degradation products if available
- The mechanism and kinetics of information of each degradation product, if available. The guidance says to determine reaction kinetics "if practicable", there by acknowledging the difficulty in cases in which the mechanism may be complex-(e.g;auto oxidation)

Other issues that may be investigated but are not explicitly requested for degradation studies are the physical and chemical stability of important crystal forms, mass balance [6,7], and the formation of stereo isomers.

Drug Product

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

- Challenge methods intended for monitoring the stability of the drug product with the degraded samples [6], or with the drug substance spiked with a mixture of known degradation products
- Isolation and characterization of significant [8], degradation products, if possible. the identify and chemical structure, procedure for isolation and purification, mechanism of formation (including order of reaction), and chemical and physical properties should be reported, if available [4], These degradation products include any drug substance-related compounds such as drug substance degradation products, drug substance –extractive degradation product, and so forth.
- Distinction between degradation products that are related to drug substances and those related to non-drug substances (21)
- Photolysis of the drug product in excess of ICH light conditions [9].
- Information requested in the submission. The available guidance [4], explicitly requests the following in the NDA documentation:
- For degradation products: Identify and structure; procedure for isolation (where applicable) and characterization: mechanism of formation, including order of reaction and physical and chemical properties
- Information about stress testing of drug substances and drug products (the guidance does not state specifically what information is required).
- More –specific guidance for the reporting of stress testing was found in FDA draft guidance documents dealing with stability [10], and method validation [11], According to these documents, the applicant should provide
- Degradation pathways [10], of the dug substance, alone and in the drug product [11].
- A discussion of the possible formation of polymeric and enantiomer substances [1], the possible formation of any stereo isomers is implied
- Data showing that neither the freshly prepared nor the degraded placebo interferes with the quantification of the active ingredient [11].
- Data from stress studies of the drug substance and drug product demonstrating the specificity of the assay and analytical procedures for degradation products [11], These data may take the form of representative instrument output (e.g., chromatograms) and/or degradation information obtained from stress studies (e.g., results of peak purity experiments performed on degraded samples).

Experimental Approach 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 [12,13]. Forced degradation studies should be conducted whenever a stability indicating method is required. Studies may need to be repeated as methods, processes. Or formulation 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 [14].

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) as the appropriate blank. A compound may not necessarily degrade under a given stress condition. No further stressing is advised in these cases [15].

Acid

Example acids include HCL or H2SO4 (0.1-1 mol/L solution). Studies should be carried out in the solution state. For certain API's 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 API's can be run as suspensions [15]. Special attention to the API, s structure should be paid when choosing the appropriate co-solvent (i.e., do not use alcohols for acidic conditions due to them re activity. Dimethylsulfoxide, acetic acid and propionic acid are quite 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.

Base

Examples bases include NaOH, LiOH, or KOH (0.1-1mol/L solution). Studies should be carried out in the solution state. For certain API's 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 API's can be run as suspensions. Glyme and 1,4-dioxane facilitate reactions in basic conditions [16]. Additionally, the sample may be heated for a defined time /temperature to accelerate degradation, depending on the API sensitivity to heat.

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 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 depend s on the free radical initiator selected. For peroxide conditions, hydrogen peroxide reagent (up to 30%) 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 excipients. Solid state stress conditions may be similarly investigated by placing the API (as is) in suitable closed containers filled with an oxygen head space versus an argon or nitrogen control head space,. 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 metals ions to solutions of API an indicate whether there is a tendency for the API to be catalytically oxidized. Iron and copper ions are routinely found in API's and formulation excipients [17], 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 [14].

Thermal /Humidity

Solid state stability can be evaluated utilizing accelerated storage temperatures in general greater than 500c and N75% 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 temperatures storage that is simulated by the study can be estimated the table in Appendix Table 2 provides a guide to that conversion. In general, an activation energy assumption of 15kcal/moles recommended,. In certain preclinical through phase 2 studies, an activation energy assumption between the recommended 15lcal/mol assumption and an aggressive assumption of 8 k cal/mol might be appropriate. Ins studies where particular concerns exist, an activation energy assumption between the recommended 15kcal/mol assumption and a conservative assumption of 12kcal/mol might be appropriate. Deviation from Arrhenius kinetics in increasingly expected above 70-80^{0}c, and the impact of this should be considered during experimental design [14].

Table 2.

Oxidative degradation		
API	Initiator	AIBN (40-600C)-Organic soluble initiator, ACVA
Radical chain		
Indicators		
(40-600c) or VA-044		
(RT-400 C)		
Water soluble initiators		
API 0.1-0.2mg/ml		
Concentration		
Initiator concentration 5-20mol%of API concentration		
Solvent Acetonitrile/water		
Temperature Ambient-600c		
Duration 5-20%degradation or 14 day maximum		

Note: Other options include exposing the sample the sample to pressured (optimum pressure of 150psi) or bubbled oxygen.

VA-044 =2,2′-Azobis(N,N)-Dimethyleneisobutyramidine)dihydrochloride

ACVA = 4,4′-Azobis(4-cyanovaleric acid)

AIBN - 2,2′-Azobisisobutyronitrile.

chart risk level to compare with correlation to shelf-life.

Photo Stability

Perform studies in accordance with ICH photo stability guidelines [18]. 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 exposure for confirmatory stability studies are an overall illumination of not less than 1.2 million lux hours and an integrated near ultra violet energy of not less than 200 W h/m2. For forced degradation studies, the samples should be exposed toatleast 2x the ICH exposure length to ensure adequate exposure of the sample,. For solution studies, acetonitrile is the co-solvent choice. Methanol can produce more artefact degradation products from methoxy radicals produced from light exposure [14] (Tables 2-4).

Table 3.

Hydrogen peroxide, formaldehyde or formic acid	Reagent concentration (choose one)	0.02-3%
Note: dependent on potential impurities in the formulation	API Concentration temperature	0.1-20mg/ml
Duration	5-20% degradation or 7 days maximum	Ambient-300c

Table 4.

API		
Transition metals	metal	Cu(II)C12 or Fe(III)C13
Note: samples should be prepared so oxygen is available	Concentration (choose one) API Concentration	0.05mM,0.1mM 0.1-20mg/mL

Note: samples should be prepared so oxygen is available	Temperature Duration	30-400C 5-20% Degradation or 14 days maximum.
Fenton conditions metal Fe(II)SO4 or Fe(II)C12		
Metal Concentration	1-5mM	
peroxide	0.3-3%	
API concentration	0.1-20mg/mL	
Temperature	30-400c	
duration	5-20%degradation or 14 days maximum	
Singlet oxygen	Photosensitizer Photosensitizer API concentration Temperature Light source Duration	Rose Bengal 0.1mM 0.1-1mg/mL Ambient-350c Xenon lamp 30-60minutes

Stress Testing of Biological or Biotechnological Products

Stress studies are also required to be conducted on biotechnological and biological products. This is a requirement under the ICH guideline Q5C [19]. The stress studies are said to be useful.

i) In determining if accidental exposures to conditions other than those proposed re deleterious to the product,

ii) For evaluating which specific test parameter may be the best indicators of product stability and

In revealing patterns of degradation.

The guideline emphasizes that the conditions of the stress study as enshrined in the tripartite stability guideline Q1A may not be appropriate for biotechnological/biological products. It is stated that 'conditions should be carefully selected on a case-by –case basis'. Accordingly, it is not claimed that the classification system and decision trees, as discussed in this paper, would apply to biotechnological/ biological drugs and their products. Unlike, chemical drugs, the bio products are particularly sensitive to factors such as temperature changes, oxidation, light, ionic strength and shear. Their main routes of decomposition also vary from chemical drugs. The bio products undergo reactions such as dimidiation, oxidation, aggregation, proteolysis, etc [19]. The exact classification system for these products need to be developed, by making a survey of the reports on intrinsic stability of these drugs (Tables 5-7). This exercise remains to be done (Appendix Table 1 and Appendix Text).

Appendix B

Recommended API and DP Degradation Conditions

API concentration - 0.1-0.1mg/ml

pH range - 1-2 ,as is solution control, 12-13

Note: based on drug pKa, other pH conditions may be appropriate.

pH adjustment - HCl (0.1-1.0M)/NaOH(0.1-1.0M) for low and high range, phosphate buffer for mid range.

Co-solvents acetonitrile or methanol (avoid methanol for API's containing a carboxylic acid, (if needed) amide,arylamide or hydroxyl group)

Temperature room temperature (elevated temperature only recommended for solution DP)

Duration - 5-20% degradation or 14days maximum

DP (not necessary if DP is not solution form)

API - Formulation dependent(usually at formulation API concentration)

Concentration

pH range +/-2 pH units around the target pH

pH adjustment HCl for low range and NaOH for high range.

Co-solvents not applicable

(if needed)

Temperature 70^0c

Duration 5-20% degradation or (3weeks maximum) see activation energy chart risk level to compare with correlation to shelf-life.

Appendix Table 1: General Protocol of API and DP Degradation Experiments.

CONDITION	API	DP		
SOLID SOLUTION/SOLID(TABLETS,SOLUTION,SUSPENSION,CAPSULES, (IV,oral blends,suspension)				
Acid/base		+		0
oxidative	0	+	+	+
photostability	+	0	+	+
thermal	+		+	+
Thermal/humidity	+		+	

Note: + = recommended; o = optical suggested for some compounds

Table 5.

DP	
Option: DP in the solid state (pressured oxygen)	
DP	Blends, tablet
Oxygen optimum:150psi (or head space of O2 at atmospheric pressure)	
Duration	1-3weeks
Temperature ambient-600C	
Temperature 30-400C	
Duration 5-20% degradation or 14 days maximum	

Table 6.

Photostability degradation	At least 2x the ICH recommendation of 1.2million lux hours.
API and DP	At least 2x the ICH recommendation of 200W-h/m2
Visible light exposure	Optional-recommended for IV,suspensions and other liquid dosage forms.
UV light exposure	1 or 2 (if using option 1 and basing the exposure on 1.2million lux hours,-exposure in the UV region will be exceeded by approximately 2.5x)
API in solution	Cover container completely with alluminium foil
ICH option	Quartz or borosilicate glass
Dark control	Note:the %transmittance of type I class A borosilicate glass at 300nm is approximately 60%.the transmittance of the sample glass after radiation. discoloration is approximately 12%.the %transmittance of type I Class B amber.
container	Borosilicate glass at 300nm is 0%(10).
	Duration instrument dependent.

Table 7.

Thermal/humidity degradation	
API and DP	
Container	open
temperature	50-700C
Relative humidity	25-75%(optional based on instrumentation and resource capabilities at the exploratory stage)

Note: Determining moisture uptake of the API may provide a narrower selection of approximate humidity conditions. If there is deliquescence, decrease temperature below the phase change. In addition, desiccators containing a saturated salt solution can be used for high potency compounds to minimize exposure or when humidity ovens are not available. A saturated NaCl solution is used for 75%relative humidity and a saturated Mgcl2 solution is used for ambient (30%) humidity (11).

Generally, free radical initiator and peroxide will produce the primary oxidation products predictive of real-time API and DP degradation. Therefore, free radical and peroxide conditions are strongly recommended at all stages of development. In early degradation discussions, ask formulators about possibility for peroxide or formic acid impurities. Problematic excipients include ethers such as polyethylene oxide, polyethylene glycol, polyvinyl pyrrolidone (PVP)/ povidone, and surfactants sorbate and tween. Maximum duration will be 2-3 weeks in exploratory. Add longer duration for late stage compounds (Appendix Table 2: relative rate factors of degradation).

Appendix Table 2: Relative rate factors of degradation.

Temperature (0c)(Ea=12kcal/mol)	Relative rate (Ea=15kcal/mol)	Relative rate (Ea=18kcal/mol)	Relative rate
25	1	1	1
30	1.39	1.52	1.65
40	2.62	3.37	4.29
50	4.78	7.1	10.5
60	8.36	14.3	24.4
70	14.2	27.7	54
80	23.5	51.8	114
90	37.7	93.3	231
100	58.9	163	451
110	89.8	277	851
120	134 457 1550		

Note: Relative rate factors of degradation (relative to 250c) assuming Arrhenius kinetics.

K=a*e-ka/Rt

Recommended activation energy assumption (15kcal/mol)

Assuming an activation energy (Ea) of 15kcal/mol, 18 months storage at 250c for example, may be simulated by 20days storage at 70^0c (since 20x27.7=554days, or approximately 18months) by 6 days storage at 90^0c or by 2 days storage at 110^0c.

Conservative assumption

Assuming an activation energy (Ea) of 12kcal/mol,18 months storage at 250c is simulated by 39days storage at 70^0c (since 39x14.2=554days, approximately 18 months) or by 9days storage at 100^0c.aggressive assumption.

Assuming an activation energy (Ea) of 18kcal/mol,18 months storage at 250c is simulated by 10 days storage at 70^0c or by 2.5days at 90^0c (since 2.5x231=578days, approximately 18 months).

Ezetimibe

Ezetimibe was subjected to different ICH prescribed stress conditions. Degradation was found to occur in hydrolytic and to some extent in the photolytic conditions. While the drug was stable to oxidative and thermal stress. The drug was particularly liable under neutral and alkaline hydrolytic conditions. A stability indicating HPLC method was developed for analysis of the drug in the presence of the degradation products [20].

Azelnidipine

We identified four degradants (Dg-A, Dg-B, Dg-C, Dg-D) of Azelnidipine to be generated under radical initiator-based oxidative conditions and proposed the mechanic pathway for their formation.2,2-Azobisisobutyronitrile was used as a radical initiator. There appeared to be two major pathways in the oxidation of the 1,4-dihydropyridine moiety. One was initiated by hydrogen abstraction from the C-4 position of the dihydropyridine ring followed by hydrogen abstraction from the N-1 position, leading to aromatisation of the di hydro pyridine ring and Dg-A generation. The other was initiated by hydrogen abstraction from the N-1 position of the dihydropyridine ring followed by oxidation and hydrolysis to yield Dg-B.furthermore, Dg-B was subjected to hydrolysis to generate Dg-C,Dg-D. It has been revealed that the rate of the Dg-B degradation was predominantly governed by the water content of the solvent used. Water participation in Dg-B degradation was proved by monitoring the incorporation of heavy oxygen atom (180) into the structure with LC-MS, which the experiment was carried out in the medium prepared with heavy oxygen water to label 180 during the hydrolysis [21].

Oseltamivir

Oseltamivir phosphate was subjected to stress degradation conditions prescribed by ICH guiselineQ1A(R2). A total of five degradation products (Os I to Os V) were generated under hydrolytic (acid and alkaline)stress conditions. Their unambiguous structural elucidation was carried out using LC-MS, LC-NMR and HR-NMR data. The first accurate masses of Os I, Os II, Os IV and Os V were determined by LC-MS/TOF. Subsequently 1H and COSY NMR studies were carried on the drug and these four degradation products using LC-NMR.the structure of Os III was elucidated after preparative isolation and purification followed by MS/TOF and HR-NMR studies. The degradation products Os II, Os IV and Os V were characterized as 4-acetamido-5-amino-3-(pentan-3-yloxy) cyclohex-1-ene carboxylic acid, 4,5-diamino-3-(pentan-3yloxy) cyclohex-1-ene carboxylic acid and ethyl 4,5-diamino-3-(pentan-3-yloxy) cyclohex-1-ene carboxylate, respectively. Os I and Os III were identified as potential isomers of Os II and the drug respectively involving N, N-acyl migration from 4-amino to 5-amino position in the ring. Two degradation pathways for all five were outlined and justified mechanistically. In silico toxicity of the drug and degradation products was also assessed using TOPKAT and DEREK software and compared [22].

Synercid

This paper describes the gradient HPLC method developed for the analysis of Synercid freeze dried powders in routine quality control and stability studies. This method was slightly extended to study the stability of Synercid solutions under the clinical conditions of administration as well as the compatibility of Synercid with other I.V drugs during Y-site simulated injection [23-25].

Atorvastatin (At) and Amlodipine (Am)

A simple rapid precise and accurate isocratic reversed-phase stability indicating HPLC method was developed and validated for the simultaneous determination of atorastatin (AT) and amlodipine (AM) in commercial tablets. This method has shown adequate separation for AM, AT from their associated main impurities and their degradation products. Degradation products produced as a result of stress studies did not interfere with detection of AT and AM and the assay can thus be considered stability-indicating [26].

Thiocolchicoside (Th) and Diclofenac Potassium (Dp)

A simple rapid and robust stability indicating RP-HPLC method has been developed and validated to measure thiocolchicoside (TH) and diclofenac potassium (DP) at single wavelength (258nm) in order to assess assay and in vitro drug release profile of drug from tablet formulation. Sample solutions were treated with HCL, NaOH of different normality and H_20_2 at different concentration. The chromatogram obtained revealed that the peak area for TH was degraded in all stressed conditions while DP peak area degraded only in the acidic condition. The percentage degradation of each compound was calculated with respect to the controlled sample. The plot of respective component % degradation against concentration of HCL, NaOH and percentage of h20 indicated apparent first order degradation of TH in all concentrations and DP only in the HCL conditioned photolytic conditions, the solution was stable [27].

Luliconazole

A stability-indicating LC method was developed and validated for the determination of Luliconazole in bulk and cream formulation. Luliconazole was exposed to acid, alkali and water hydrolysis, oxidation effect by hydrogen peroxide, dry heat and photolytic conditions. Full factorial designs were used during forced degradation experiment the factors/combination of factors which were most likely to effect degradation of Luliconazole under various conditions were identified and further were optimized using surface response curve drug was found to be stable under wet heat and dry heat conditions, but substantial degradation was observed under acid, alkali, oxidative and photolytic conditions. There was no interference of excipients and degradation products in the determination of active pharmaceutical ingredient [28].

Lumefantrine

Forced degradation studies were carried out on Lumefantrine by subjecting it to stress conditions (hydrolysis(acid,base),oxidation, photolysis and thermal degradation) and degraded samples were further analyzed by using this method. Major degradation was observed in alkaline and oxidative conditions. The drug was quite stable under the other stress conditions investigated. The degradation products were well resolved from main analyte peak. Thus the method proved to best stability indicating [29].

Artemether

An accelerated forced degradation study on the Artemether significant degradation was observed when artemether sample solution exposed to acid at room temperature, base and peroxide. Rapid degradation observed when Artemether samples were exposed to acid at 600C and UV-light. The degraded samples show a decrease in assay value, indicating that the assay method is stability indicating [30].

Ranitidine Hydrochloride

In this study, ranitidine hydrochloride stock solution was kept in the dark at 80^0C for 30 days and were analyzed at different times (every day).it has been that repeatable peak currents of ranitidine

hydrochloride stock solutions occurred up to 15 days and after that the peak current decreased significantly. So, the solutions were found to be stable for 30days [31].

Nicardipine

A forced degradation study of nicardipine in bulk and in its tablet form was conducted under the conditions of hydrolysis, oxidation, and photolysis in order to develop a rapid and sensitive stability indicating LC-UV method for qualification of nicardipine. Nicardipine was found stable in acidic buffer up to 48 hours while in alkaline buffer found degraded. The degradation was not observed for Nicardipine sample during stress conditions tested. The mass balance of nicardipine in test sample was close to 100% and moreover, the unaffected assay of nicardipine in presence of degradation product confirms the stability indicating power of the method [32].

- Acid hydrolysis- no degradation
- Base hydrolysis degradation observed
- Oxidation degradation observed
- UV (254nm) degradation observed

Desvenlafaxime

Desvenlafaxime stock solution was subjected to acid and alkali hydrolysis, chemical oxidation and dry heat degradation. The drug was found to be susceptible to base hydrolysis and a developed method was found to give good separation between the pure drug and the degraded product. Stress degradation study using acid and alkali hydrolysis, chemical oxidation and dry heat degradation was carried out and interference of the degradation products was investigated. Oxidative stress degradation sample showed degradation peak at rc retention time 4.12 and4.50min.the peaks around 0.2-1.5 min in the chromatogram is because of the impurities present in the hydrogen peroxide.dry heat degradation study revealed that there is no degradation peak for DVX. The drug was found to be stable [33].

Alprazolam and Propranolol

The method was validated by subjecting the drugs to forced decomposition under the hydrolysis, oxidation, photolysis and thermal stress conditions prescribed in international conference on harmonization.

Acidic condition:- The individual drug and their combination were heated in HCL for various conditions compared with the APL,PRL was more susceptible to the degradation process, about 20-30% degradation of PRP was observed. Whereas minute degradation of API was seen.

- Degradation In Alkali

PRP underwent alkali hydrolysis, but the rate of hydrolysis was slower than that under acidic conditions. The degradation patterns was similar to those seen under acidic condition.

- Oxidative Condition

Both the drugs were highly liable to oxidative hydrolysis in 3%H2O2, PRP was competitively more liable than ALP. Around 48% degradation was observed in the case of PRP and 15-20% in the case of ALP.

- Thermal Degradation

Both the drugs relatively stable when exposed to dry heat at 800 C for 48 hours. The percentage of both drugs remaining after 48 hours of exposure to dry heat were in the range of 95-98%

- Photolytic Conditions

Both the drugs relatively stable when exposed to UV for 36hours.the percentage of both drugs remaining after 36hours of exposure to UV were in the range of 95-98% [34].

letrozole

The aim of the present work is to develop and validate a stability indicating liquid chromatographic method for the assay of letrozole in bulk and tablet dosage in presence of its degradation products and to establish the possible stable condition using related compound A standard. forced degradation was attempted using light, heat, acid, base, oxidation, reduction and hydrolysis, base hydrolysis, oxidation, reduction and hydrolysis). The results of the forced degradation of the sample drug for 2 hours under the above-mentioned conditions indicated that the recovery of the drug was more than 80% in all the conditions except in the case of base hydrolysis condition. There was no letrozole peak in the base hydrolysis chromatogram indicating a major degradation [35].

Cilostazol

This study was carried out by subjecting the drug to stress conditions like acid, alkaline and oxidizing agent treatment. However, no attempts were made to isolate the degradation products from the drug. The ability of the method to trace the degradation of the drug which is evident by the presence in the shift in the UV-absorption spectra of the drug is an indicator for the method specificity for the drug in the given experimental conditions and also provides information on its stability indicating property [36].

Aspirin (ASP)

Atorvastatin (ATV), Ramipril (RMP) and Metoprolol (MTP): A novel sensitive and precise UHPLC method has been developed and

validated for the simultaneous determination of all the active components of Aspirin (ASP) atorvastatin(ATV), ramipril(RMP) and metoprolol(MTP) in tablet dosage form In the presence of degradation products. forced degradation f individual as well as combination of all drugs substances components of polypill was conducted in accordance with ICH guidelines. Acidic, basic, neutral and oxidative hydrolysis, thermal stress and photolytic degradation were used to assess the stability -indicating power of the method. The mixture of all the four drug components was exposed to 0.1N HCl at 100o C for 1hour. ASP and ATV showed considerable degradation with time in 0.1N HCl with the formation of salicylic acid (SA) as the major degradants, when treated under basic consideration ASP and RMP has shown significant degradation immediately in 0.1N NaOH with ASP is converted into salicylic acid (SA). under oxidation conditions, ASP and ATV has shown considerable degradation by the treatment of 5hydrogen peroxide. All the four drug combinations were exposed to light for an overall illumination of 1.2 million lux hours and an integrated near ultraviolet energy of 200-watt hours/sq meter. (w/n hr) (in photo stability chamber. Major degradation observed with ATV. The proposed method is applied for the assay analysis of 3different batches of the zyead. The assay results obtained were within the specification limit. The assay of polyprill is unaffected in the presence of degradation impurities confirming the stability indicating power of the developed method. The stability indicating nature of the method was further confirmed by injecting a 3month accelerated stability sample and observed that all the degradants were well separated from the main components [37].

Indapamide and telmisartan

A simple, precise, accurate RP-HPLC method has been developed for the estimation of indapamide and telmisartan in bulk and in capsule formulation. In this study, the products formed after forced decomposition studies were resolved from the bulk drug response [38]. From the peak purity profile studies. It was confirmed that the peak of degradation product was not interfering with the peak of drugs. It confirms that peak for degradation product of drug can be resolved from the drug peak by this method (42).

Gilbenclamide

A forced degradation study on gilbenclamide was performed under conditions of hydrolysis, oxidation, dry heat, and photolysis and a high-performance column liquid chromatographic ultraviolet (HPLC-UV) method was developed to study degradation behavior of the drug under the forced conditions [39]. The degradation products formed under different forced conditions were characterized through isolation and subsequent under infrared/nuclear magnetic resonance/mass spectral analyses, or through HPLC/mass spectroscopic (HPLC/MS) studies. The drug degraded in o.1M HCL and water at 85C to a major degradation product,5-chloro-2-methoxy-N-2-(4-sulfamoylphenyl) ethyl benzamide (III),and to a minor product,1-cyclohexyl-3-[(4-(2-amino ethyl)-phenyl]sulphonyl urea IV upon prolonged heating in the acid, the minor product IV disappeared resulting in the formation of 5-cloro-2-methoxy-benzoic acid (II) and an unidentified product(I).heating of the drug in 0.1MNaOH at 85degreecentigrade yielded II and IV as the major products and I and III as the minor products.(43)

Mirtazapine

Mirtazapine was subjected to stress degradation under different conditions recommended by the international conference on harmonization (ICH). Mirtazapine drug was found to degrade under acidic condition. When Mirtazapine was treated with 0.1N HCL and sample was withdrawn after an interval 1,3,5 and 8 hours, it undergoes degradation. Upon the treatment of Mirtazapine with 3% H2O2 at normal condition no additional peaks were detected but after refluxing it [40], it undergoes degradation. When Mirtazapine was exposed to light source, the Mirtazapine content exhibited slight decrease and an additional peak was also detected. When Mirtazapine was exposed to heat there was no change in the peak area for Mirtazapine. No additional degradation peaks were detected. When the marketed tablets without primary packaging were subjected to milder stress conditions there was no peak for the product of degradation. (44)

Rebamipide

Rebamipide (drug and drug product) solutions were exposed to acid and alkali hydrolysis, thermal stress, oxidation by hydration peroxide and photo degradation. In addition, acid and alkali hydrolysis was performed using a microwave oven. From the preliminary experiments no degradation was found under acid and water hydrolysis using microwave oven as well as with conventional method of heating. Hence, it was decided not to proceed further with these conditions. But for alkali hydrolysis with microwave oven as well as conventional method of heating, degradation has been obtained. Therefore, it was decided to apply an experimental design for it to get optimum degradation conditions [41]. The drug was found to be stable to acid hydrolysis, oxidative, wet heat conditions, and photolytic conditions as no decrease in peak area of drug was observed with no secondary peaks. The alkaline hydrolysis leads to the breakdown of amide (C-N) bond which leads to the formation of two degradation products: p-chloro benzoic acid(I)and 3-(2-oxo-1,2-dihydroquinolin-4-yl) alanine (II). when standard p-chlorogenic acid was chromatographed with same chromatographic conditions. It has been observed that retention time of Deg 2 matched with retention time of standard p-chloro benzoic acid (4.06min for Deg 2 and 4.12min for standard p-chlorobenzoic acid). hence it has been confirmed that one of the degradation product was p-chlorobenzoic acid (Deg 2) and other might be 3-(2-oxo-1,2-dihydroquinolin-4-yl) alanine (Deg 1)(45).

Summary

Forced degradation studies of new drug substances and drug products are essential to help develop and demonstrate specificity of stability-indicating methods and to determine the degradation pathways and degradation products of the active ingredients. They also can be useful in the investigation of the chemical and physical stability of crystal forms, the stereo chemical stability of the drug substance-related degradation products needed for method validation often emerge from these studies. Knowledge gained from these studies can be used to guide formulation development and improve manufacturing and packaging processes. For marketing applications, current FDA and ICH guidance recommends inclusion of the results, including chromatograms of stressed samples, demonstration of the stability-indicating nature of the analytical procedures and the degradation pathways of the drug substance in solution, solid state and drug product. The structures of significant degradation products and the associated procedures for their isolation and/or characterization also are expected to be included in the filling. The experimental protocol for degradation studies will depend on the active ingredients and formulation involved because the chemistry of each compound is different. A target of the littlest of 10% degradation of the active ingredient or exposure to energy in slight excess of accelerated storage is recommended. A compound may not necessarily degrade under a given stress condition. No further stressing is advised in these cases.

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