Forced degradation studies – comparison between ICH, EMA, FDA and WHO guidelines and ANVISA's resolution RDC 53/2015

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List of Abbreviations

ANVISA	National Health Surveillance Agency (in Portuguese Agência Nacional de Vigilância Sanitária)
Art.	Article
API	Active Pharmaceutical Ingredient
ATD	Average Daily Dosage
CGMP	Current Good Manufacturing Practices
CP	Public Consultation (in Portuguese Consulta Pública)
CTD	Common Technical Document
DMF	Drug Master File
EMA	European Medicines Agency
FDA	Food and Drug Administration, USA
FDC	Fixed-Dose Combination
FPP	Finished Pharmaceutical Product
HPLC	High Performance Liquid Chromatography
ICH	International Conference on Harmonization
IND	Investigational New Drug Application
JP	Japanese Pharmacopoeia
LoD	Limit of Detection
LoQ	Limit of Quantification
PDA	Photodiode Detector Array
QOS-PD	Quality Overall Summary - Product Dossiers
RDC	Board Resolution Collegiate (in Portuguese Resolução de Diretoria Colegiada)
RRF	Relative Response Factor
RRT	Relative Retention Time
RH	Relative Humidity
RT	Room Temperature
USP	US Pharmacopoeia
UV	Ultraviolet
WHO	World Health Organization
EPAR	European Public Assessment Report
WHOPAR	World Health Organization Public Assessment Report

1. Introduction and scope

Forced degradation is an exposure of the drug substance or drug product to different stress conditions (more severe than accelerated conditions) [1] which results in relevant degradation products. Purposefully used forced degradation is a useful tool in predicting drug stability. The drug stability is a critical parameter and has an impact on purity, potency, and safety of the drug product. For instance, changes in drug stability can result in lower doses or toxic degradation products. Therefore it is fundamental to know the behavior and the purity profile of a drug under various conditions [2].

Currently several guidelines provide recommendations and guidance on forced degradation studies, but none of the guidelines gives detailed, complete and clear instructions or definitions regarding the individual aspects e.g. exact conditions or exposure times. This leads to uncertainty and disagreement between the pharmaceutical companies resulting in different approaches when conducting forced degradation studies.

In the past years one of the national regulatory agencies - the Brazilian National Health Surveillance Agency (ANVISA) - has dedicated themselves to deal with the topic “forced degradation”. ANVISA has taken over a pioneering task in establishing further requirements and guidance in comparison to what is currently available.

In 2013 ANVISA published resolution RDC 58/2013 and introduced new standards for reporting, identification and qualification of degradation products in drug products [3]. To enable the companies to comply with the new requirements ANVISA additionally issued a degradation products guide, the revised document CP 68 [4]. The guide is intended to promote the views of ANVISA regarding the degradation profile and the testing procedures for the identification and qualification of degradation products.

According to the deadline for the Collegiate Board Resolution (RDC), the resolution RDC 58/2013 was expected to come into force end of December 2015.

But on December 4th 2015 ANVISA revoked RDC 58/2013 and published instead an updated version of this resolution: Resolution RDC 53/2015 [5]. Resolution RDC 53/2015 includes updated standards on different aspects and topics of forced degradation to which the pharmaceutical companies have to comply. For all new concentration or new dosage form inclusions the new resolution became valid on December 23, 2015. For medicines which are already registered in Brazil the resolution RDC 53/2015 includes different timeliness for the implementation.

The aim of this master thesis is:

- To provide a general overview on the topic forced degradation and on the current available regulatory guidance (ICH including EMA, FDA and WHO)
- To give an overview on the requirements of resolution RDC 53/2015 and provide a comparison between the new standards laid down in ANVISA's resolution RDC 53/2015 and the currently available regulatory standards
- To start a discussion on the differences and similarities as well as the challenges and critical points for the pharmaceutical companies to meet the new requirements
- To discuss the content of a protocol describing the degradation profile for products, which is requested by ANVISA to be provided by the pharmaceutical companies in the content of this resolution

2. Forced degradation studies

2.1 Terms for forced degradation

Internationally different terms are used for the description of forced degradation. Even within the ICH guidelines more than one term for the description of forced degradation is used e.g. ICH Q1A [1] uses the term 'stress testing', while ICH Q1B [7] uses the term 'forced decomposition'.

2.2 Purpose of forced degradation testing

According to the ICH guideline Q1A, section 2.1.2 the purpose of stress testing for the new drug substances is as follows [1]:

"Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used. The nature of the stress testing will depend on the individual drug substance and the type of drug product involved".

In summary, from the regulatory perspective, forced degradations studies are performed [1]:

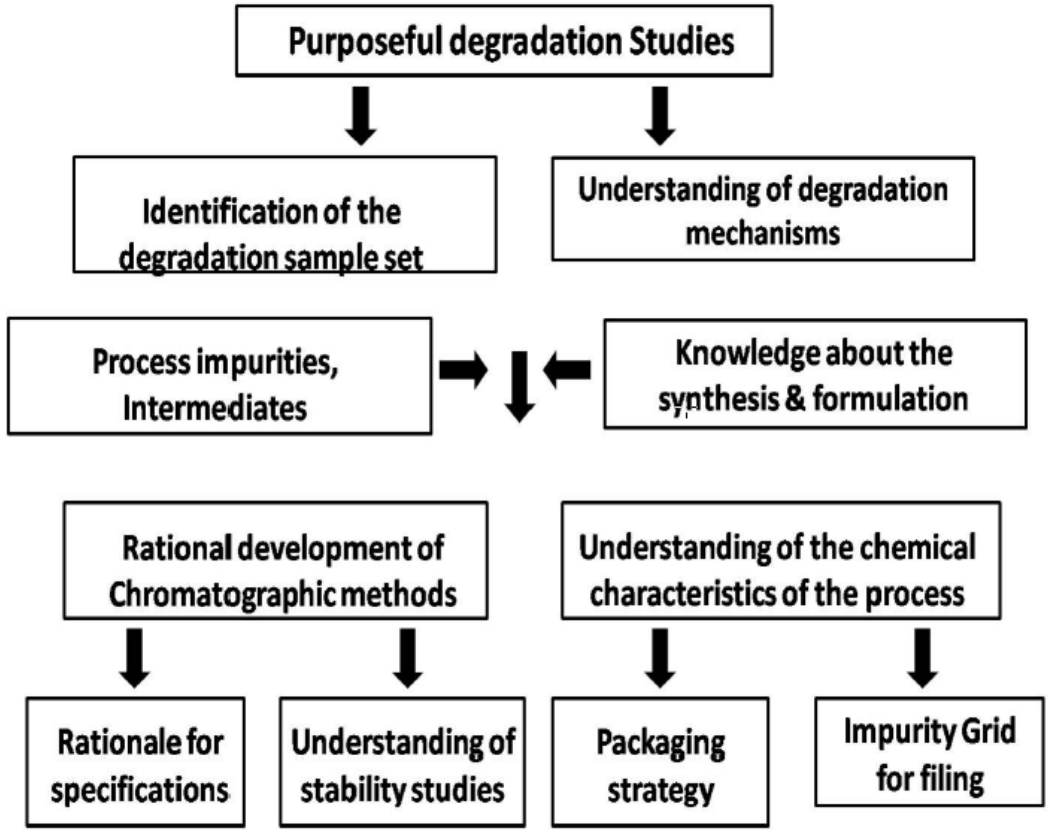
- To identify possible degradation products
- To establish degradation pathways and intrinsic stability of the drug molecule
- To validate stability-indicating analytical procedures

When looking beyond ICH guidelines and looking into the literature and the current trends for forced degradation studies, the initial purpose of forced degradation studies is to understand drug molecule chemistry [8], to investigate stability-related properties of an API and to develop an understanding of the degradation products and pathways [9]. In following a selection of purposes for performing forced degradation studies is listed [2, 8, 9, 10, 11, 12]:

- "to elucidate the structure of the degradation products"
- "to develop and validate a stability-indicating analytical method"
- "to identify impurities related to drug substances or excipients"
- "to generate more stable formulations"
- "to distinguish degradation products in formulations that are related to drug substances from those that are related to non-drug substances (e.g., excipients)"
- "to solve stability-related problems (e.g., mass balance)"
- "to generate a degradation profile that mimics what would be observed in a formal stability study under ICH conditions"
- "to facilitate improvements in the manufacturing process and formulations in parallel with accelerated pharmaceutical studies"
- "to choose the correct storage conditions, appropriate packaging and better understanding of the potential liabilities of the drug molecule chemistry"
- "to facilitate improvements in the manufacturing process and formulations in parallel with accelerated pharmaceutical studies"

Figure 1 shows the importance of forced degradation studies with respect to current pharmaceutical scenarios [13].

Figure 1: Importance of forced degradation in pharmaceuticals



3. Regulatory overview

3.1 ICH guidelines - regulatory overview

Until today ICH (The International Committee for Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use) [6] has achieved harmonization in many areas of quality e.g. in conducting of stability studies or in providing definition of relevant thresholds for impurity testing. ICH has published several guidelines which have been discussed, agreed upon and adopted by the regulatory authorities of the ICH regions United States, Europa and Japan [6]. When it comes to the topic "forced degradation" the most ICH guidelines emphasize the importance of conducting forced degradation studies, but provide only very general and limited information on the experimental stress conditions and only general guidance on how to conduct forced degradations studies. For example, the guidelines do not provide specific information and recommendations on the stress conditions e.g. pH, temperature ranges, specific oxidizing agents, or conditions to use.

Furthermore, the guidelines mostly refer to new drug substances and drug products and do not refer to drug substances and clinical development.

Following ICH guidelines are in place and applicable when searching for guidance with regard to conducting forced degradation studies:

- ICH Q1A – Stability Testing of New Drug Substances and Products [1]
- ICH Q1B – Photostability Testing of New Drug Substances and Products [7]
- ICH Q2B – Validation of Analytical Procedures: Methodology [14]
- ICH Q3A – Impurities in New Drug Substances [15]
- ICH Q3B – Impurities in New Products [16]
- M4Q(R1) – The common Technical Document (CTD): Quality [17]

3.1.1 ICH Q1A – Stability Testing of New Drug Substances and Products

ICH Q1A guideline understands under stress testing studies of a drug substance "studies which are undertaken to elucidate the intrinsic stability of the drug substance. Such testing is part of the development strategy and is normally carried out under more severe conditions than those used for accelerated testing" [1].

Studies undertaken to assess the effect of severe conditions on the drug product include photostability testing and specific testing on certain products, (e.g., metered dose inhalers, creams, emulsions, refrigerated aqueous liquid products) [1].

Section 2.1.2 of the Q1A guideline (Stress Testing) emphasizes that stress testing of the drug substance can help to identify the likely degradation products and can help to establish the degradation pathways and the intrinsic stability of the molecule [1].

Furthermore, the stress testing plays a significant role regarding validation of the stability indicating power of the analytical procedures used. The guideline states that the stress testing depends on the individual drug substance and the type of the involved drug product. Following recommendations for drug substances and drug products on the test conditions for performing forced degradation studies are given [1]:

- Stress testing is likely to be carried out on a single batch of the drug substance
- Stress testing should examine the effects of the temperature in (10°C increments (e.g., 50°C, 60°C, etc.) above that for accelerated testing)

- Testing in solution should also be performed across a wide pH range either as a solution or suspension. These samples are then used to develop a stability-indicating method.
- Humidity should be examined at (e.g., 75% RH or greater)

With regard to oxidation, and photolysis on the drug substance no specific recommendations are provided. There is only a general advice to perform these tests when appropriate. Photostability testing is adjudged to be an integral part of stress testing. For the standard conditions for photostability testing ICH Q1A [1] refers to ICH Q1B [7].

Regarding the degradation products formed during forced degradation studies ICH Q1 states that it may not be necessary to examine specifically for certain degradation products if it has been demonstrated that they are not formed under accelerated or long term storage conditions [1].

3.1.2 ICH Q1B – Photostability Testing of New Drug Substances and Drug Products

ICH Q1B guideline on "Photostability Testing of New Drug Substances and Drug Products" distinguishes between forced degradation testing and confirmatory testing for drug substances". Table 1 provides definitions for both studies [7].

Table 1: Definitions for forced degradation testing and confirmatory studies

Forced degradation studies	**Confirmatory studies**
"Studies undertaken to degrade the sample deliberately. These studies, which may be undertaken in the development phase normally on the drug substances, are used to evaluate the overall photosensitivity of the material for method development purposes and/or degradation pathway elucidation" [7].	"Studies undertaken to establish photostability characteristics under standardized conditions. These studies are used to identify precautionary measures needed in manufacturing or formulation and whether light resistant packaging and/or special labeling is needed to mitigate exposure to light. For the confirmatory studies, the batch (es) should be selected according to batch selection for long-term and accelerated testing which is described in the parent guideline" [7].

ICH Q1B guideline outlines that the purpose of forced degradation testing studies is to evaluate the overall photosensitivity of the material for method development purposes and/or degradation pathway elucidation. This testing may involve the drug substance alone and/or in simple solutions/suspensions to validate the analytical procedures. The guideline provides recommendations with regard to the approaches how to assess the photostability of drug substances and drug products to be used in development of stability indicating studies [7].

Forced degradation conditions for the drug substance are described in section 2 of the guideline and for the drug product in section 3. Specific exposure levels for forced degradation studies are not defined, although they can be greater than that specified for confirmatory (stability) testing. The actual design of photostability studies is left to the applicant; however, it is mentioned that scientific justification is required for cases where light exposure studies are terminated after a short time, e.g., where excessive degradation is observed [7].

The guideline indicates that photostability testing can be introduced to solids or solutions and suspensions. These samples are used to develop a stability indicating method. The forced degradation studies should be designed to provide suitable information to develop and validate test methods for the confirmatory studies. These test methods should be capable of resolving and detecting photolytic degradants that appear during the confirmatory studies [7]. It is further stated that when the results of these studies are evaluated, it is important to recognize that they form part of the stress testing and are not therefore designed to establish qualitative or quantitative limits for change [7].

Similar as noted in the Q1A guideline, the Q1B guideline also indicates that under forcing conditions, decomposition products may be observed that are unlikely to be formed under the conditions used for confirmatory studies. This information may be useful in developing and validating suitable analytical methods. If in practice it has been demonstrated they are not formed in the confirmatory studies, these degradation products are not need to be further examined [1, 7].

3.2 ICH Q2B – Validation of Analytical Procedures: Methodology

The ICH Q2B guideline provides guidance and recommendations on how to perform validation of analytical procedures. Part II, section 1.2.2 (Impurities Not Available)

includes a recommendation for using samples from forced degradation studies. The samples should be stored under relevant stress conditions e.g. light, heat, humidity, acid/base hydrolysis and oxidation in order to prove specificity in case of unavailability of impurity or degradation product standards [14]. Furthermore, under the validation parameter "specificity" the ICH Q2B guideline understands the ability "to unequivocally assess the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc." [14].

3.2.1 ICH Q3A Impurities in New Drug Substances/ ICH Q3B Impurities in New Products

ICH Q3A guideline "Impurities in New Drug Substances" [15] and ICH Q3B guideline "Impurities in New Products" [16] require identification of each impurity under the consideration of as well as the safety as also the chemistry aspects. Both guidelines advise that "when identification of an impurity is not feasible, a summary of the laboratory studies demonstrating the unsuccessful effort should be included in the application" [15, 16].

Under section 3 (Analytical Procedures) ICH Q3B provides some recommendation on stress conditions. "In particular, analytical procedures should be validated to demonstrate specificity for the specified and unspecified degradation products. As appropriate, this validation should include samples stored under relevant stress conditions: light, heat, humidity, acid/base hydrolysis, and oxidation" [16].

3.2.2 M4Q(R1) – The Common Technical Document for the Registration of Pharmaceuticals for Human Use: Module 3: Quality

Following guidance is provided in M4Q(R1) "The Common Technical Document for the Registration of Pharmaceuticals for Human Use: Module 3: Quality" under section 3.2.S.7.1 (Stability Summary and Conclusions): "The types of studies conducted, protocols used, and the results of the studies should be summarized". The summary should include results, for example, from forced degradation studies and stress conditions, as well as conclusions regarding storage conditions and retest date or shelf life, as appropriate" [17].

Section 3.2.S.7.3 (Stability Data) states the following "results of the stability studies (e.g., forced degradation studies and stress conditions) should be presented in an appropriate

format such as tabular, graphic, or narrative. Information on the analytical procedures used to generate the data and validation of these procedures should be included" [17].

3.3 EMA guidelines - regulatory overview

Requirements for stress tests are also mentions in some of the EMA guidelines e.g. is following stated under stability section 4.7, 3.2.S.7.1 (Stability Summary and Conclusions) of the "Draft guideline on the Chemistry of Active Substances": "The types of studies conducted, protocols used, and the results of the studies should be summarized. The summary should include results, for example, from forced degradation studies and stress conditions (light stress, higher temperature, etc.), as well as conclusions with respect to storage conditions and retest date or expiry date as appropriate" [18].

Furthermore, the note for guidance on "Stability Testing of Existing Active Substances and Related Finished Products" [19] mentions under the section on 2.1.2 (Stress Testing) the following: "stress testing helps to determine the intrinsic stability of the molecule by establishing degradation pathways in order to identify the likely degradation products and to validate the stability indicating power of the analytical procedures used. For an active substance the following approaches may be used: (a) when an active substance is described in an official pharmacopoeial monograph (European Pharmacopoeia or the Pharmacopoeia of a European Union Member State) no data are required on the degradation products if they are named under the headings ''purity test'' and/or ''transparency statement''; in this case no stress testing is required; (b) when available, it is acceptable to provide the relevant data published in the literature to support the proposed degradation pathways; (c) when no data are available in the scientific literature, including official pharmacopoeias, stress testing should be performed" [19].

The guideline on the "Requirements to the Chemical and Pharmaceutical Quality Documentation Concerning Investigational Medicinal Products in Clinical Trials" states in section 2.2.1.S.7 (Stability) that "parameters known to be critical for the stability of the drug substance need to be presented, i.e., chemical and physical sensitivity, e.g. photosensitivity, hygroscopicity. Furthermore it is indicated that the potential degradation pathways should be described [20].

Moreover, the guideline on "Stability Testing for Applications for Variations to a Marketing Authorization" provides following recommendations: "the scope and design of

the stability studies for variations and changes are based on the knowledge and experience acquired on the active substances and finished products. The available information must be taken into account such as: a) for active substances: the stability profile including the results on stress testing, if applicable" [21].

3.4 FDA guidelines - regulatory overview

Refer to the FDA guideline "Guidance for industry Q1B Photostability Testing of New Drug Substances and Products" [22] "forced degradation testing studies are studies undertaken to degrade the sample deliberately". The guideline indicates that studies on the drug substances are normally conducted in the development phase. These studies are used to evaluate the overall photosensitivity of the material for method development purposes and/or degradation pathway elucidation [22]. The guideline differentiates between forced degradation testing and confirmatory testing, whereby the purpose of forced degradation testing studies is to evaluate the overall photosensitivity of the material for method development purposes and/or degradation pathway elucidation [22]. Furthermore, it is emphasized that forced degradation studies may also be used for validation purposes of suitable analytical methods using different exposure conditions. Regarding how to conduct the degradation tests the guideline refers to the applicant's discretion, although the exposure levels used should be justified. The aim of the forced degradation studies is to provide suitable information on development and validation of test methods for the confirmatory studies [22]. Regarding the degradation products which in practice are not formed in confirmatory studies the guideline says that these degradation products do not need be examined further [22].

When looking into "Questions and answers on current good manufacturing practice Good Guidance Practices Level 2 Guidance - Labaratory Controls" of the CGMP regulations, drug product stress testing (forced degradation) may not be necessary when the routes of degradation and the suitability of the analytical procedures can be determined through use of the following [23]:

- Data from stress testing of drug substance
- Reference materials for process impurities and degradants
- Data from accelerated and long-term studies on drug substance

- Data from accelerated and long-term studies on drug product

Section 211.165(e) of the CGMP regulations states that the accuracy, sensitivity, specificity, and reproducibility of test methods shall be established and documented. Further, section 211.166(a)(3) indicates that stability test methods should be reliable, meaningful, and specific, which means that the content of active ingredient, degradation products, and other components of interest in a drug product can be accurately measured without interference, often called "stability-indicating" [23]. It is not specified what techniques or tests should be used to ensure that one's test methods are stability-indicating. However, evaluating the specificity of the test methods during forced degradation studies (i.e., exposing drug to extremes of pH, temperature, oxygen, etc.) of drug substance and drug product is often necessary to ensure that stability test methods are stability-indicating. But it is described that in certain circumstances conducting a forced degradation study of just the drug substance may be sufficient to evaluate the stability-indicating properties of a test method [23].

Generally, when determining whether it is necessary to conduct forced degradation studies of the drug product, the specificity of the test method should be evaluated for its ability to assay drug substance, degradants, and impurities, in the presence of each other, without interference. The evaluation also should provide assurance that there isn't a potential for interaction between drug substance, degradants, impurities, excipients, and container-closure system during the course of the shelf-life of the finished drug product Last, the rationale for any decision made concerning the extent of the forced degradation studies conducted as well as the rationale for concluding that a test method is stability-indicating should be fully documented [23].

Some guidance on how the test should be performed is provided in the "FDA Guidance for Industry, INDs for Phase 2 and 3 Studies - Chemistry, Manufacturing, and Controls Information" [24]. The guidance emphasizes that stress studies should be done in different pH solutions, in the presence of oxygen and light, and at elevated temperatures and humidity levels to determine the stability of the drug substance. Furthermore it is stated that stress studies are conducted on a single batch and the achieved stress test results should be summarized and submitted in an annual report [24].

The FDA "Guidance for Industry, INDs for Phase 2 and 3 Studies of Drugs, Including Specified Therapeutic Biotechnology-Derived Products" [25] provides information and guidance on early stress testing.

3.5 Pharmacopoeia requirements- regulatory overview

No information regarding forced degradation was identified in the European Pharmacopoeia (EP) [26]. In following limited information as derived from the United States Pharmacopoeia (USP) [27] and the Japanese Pharmacopoeia (JP) [28] is provided.

3.5.1 USP Pharmacopoeia

In the general chapter <1225> "Validation of Compendia Procedures" [27] it is stated that "if an impurity or degradation product standards are unavailable, specificity may be demonstrated by comparing the test results of samples containing impurities or degradation products to a second well-characterized procedure (e.g., a pharmacopeial or other validated procedure) [27]. These comparisons should include samples stored under relevant stress conditions (e.g., light, heat, humidity, acid/base hydrolysis, and oxidation). In the case of the assay, the results should be compared; in the case of chromatographic impurity tests, the impurity profiles should be compared" [27].

3.5.2 JP Pharmacopoeia

Following statement was identified in the Japanese Pharmacopoeia, chapter 2.3.2 [28]: "It should be confirmed that the proposed analytical procedure can identify an analyte or that it can accurately measure the amount or concentration of an analyte in a sample. The method to confirm the specificity depends very much upon the purpose of the analytical procedure". For example, the specificity may be assessed by comparing analytical results obtained from a sample containing the analyte only with results obtained from samples containing excipients, related substances or degradation products, and including or excluding the analyte [28]. Furthermore, the following is stated: "If reference standards of impurities are unavailable, samples that are expected to contain impurities or degradation products may be used (e.g. samples after accelerated or stress tests)" [28].

3.1 World Health Organization

The World Health Organization (WHO) guideline on "Stability Testing of Active Pharmaceutical Ingredients and Finished Pharmaceutical Products" [29] is in accordance to ICH Q1A and includes similar requirements, as mentioned in ICH Q1A [1] for new drugs.

However, for well-established APIs WHO provides following important information [29]: "For an API the following approaches may be used, when available, it is acceptable to provide the relevant data published in the scientific literature to support the identified degradation products and pathways; and when no data are available, stress testing should be performed."

In the draft WHO guideline on "Submission of Documentation for a Multisource (Generic) Finished Pharmaceutical Product: Quality Part" in section 3.2.S.7.1 (Stability Summary and Conclusions) following information is provided [30]: "The types of studies conducted, protocols used, and the results of the studies should be summarized. The summary should include results, for example, from forced degradation studies and stress conditions, as well as conclusions with respect to storage conditions and retest date or shelf life, as appropriate." Furthermore, in same section under Stress Testing following information is included [30]: "As outlined in the ICH Q1 guidance document, stress testing of the API can help to identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used. The nature of the stress testing will depend on the individual API and the type of FPP involved. Stress testing may be carried out on a single batch of the API".

Examples of typical stress conditions are provided in the WHO Technical Report Series, No. 953, Annex 2, Section 2.1.2: ''A typical set of studies of the degradation paths of an active pharmaceutical ingredient'' and in the WHO Technical Report Series, No. 929, Annex 5 [30]. The objective of stress testing is not to completely degrade the API, but to cause degradation to occur to a small extent, typically 10-30% loss of active by assay when compared with non-degraded API. This target is chosen so that that some degradation occurs, but not enough to generate secondary products [30]. For this reason, the conditions and duration may need to be varied when the API is especially susceptible to a particular stress factor. In the total absence of degradation products after 10 days, the API is considered stable under the particular stress condition [30].

The tables in the QOS-PD template should be used to summarize the results of the stress testing and should include the treatment conditions (e.g. temperatures, relative humidities, concentrations of solutions, durations) and the observations for the various test parameters (e.g. assay, degradation products). The discussion of results should high light whether mass balance was observed [30].

Photostability testing should be an integral part of stress testing. The standard conditions are described in ICH Q1B. If ‘‘protect from light’’ is stated in one of the officially recognized pharmacopoeia for the API, it is sufficient to state ‘‘protect from light’’ on labeling, in lieu of photostability studies, when the container closure system is shown to be light protective. When available, it is acceptable to provide the relevant data published in scientific literature (inter alia WHOPARs, EPARs) to support the identified degradation products and pathways.” [30].

In section 3.2.P.8.1 under (Stress Testing) following is stated for drug products [30]: “As outlined in the WHO stability guideline, photostability testing should be conducted on at least one primary batch of the FPP if appropriate. If ‘‘protect from light’’ is stated in one of the officially recognized pharmacopoeia for the API or FPP, it is sufficient to state ‘‘protect from light’’ on labeling, in lieu of photostability studies, when the container closure system is shown to be light protective. Additional stress testing of specific types of dosage forms may be appropriate (e.g. cyclic studies for semi-solid products, freeze-thaw studies for liquid products)”.

The WHO guideline for Registration of “Fixed-Dose Combination Medicinal Products” furthermore includes a table that lists typical stress conditions for pre-formulation stability studies see Table 2 [30].

Table 2: Typical stress conditions in pre-formulation stability studies [30]

Stress factor	Conditions	Concentration of API[a]	Time
Heat	60 °C	1 : 1 with diluent[b]	1–10 days
Humidity	75% relative humidity or greater	Solid state	1–10 days
Acid	0.1 N hydrochloric acid	2 : 1 in 0.1 N hydrochloric acid	1–10 days
Base	0.1 N sodium hydroxide	2 : 1 in 0.1 N sodium hydroxide	1–10 days
Oxidation	3% hydrogen peroxide	1 : 1 in 3% hydrogen peroxide	1–3 hours
Photolysis	Metal halide, mercury, xenon or ultraviolet-B fluorescent lamp	1 : 1 with diluent[b]	1–10 days
Metal ions (optional)	0.05 M Fe_{2+} or Cu_{2+}	1 : 1 with solution of metal ions	1–10 days

[a] When testing degradability of APIs in combination, the APIs should be in the same ratio as in the FDC-FPP.
[b] In each case, the diluent is either an excipient or all excipients in the formulation in the same ratios as in the formulation. Other ratios of diluent may also be appropriate, for example the approximate ratio in which the drug and excipients will be used in a formulation.

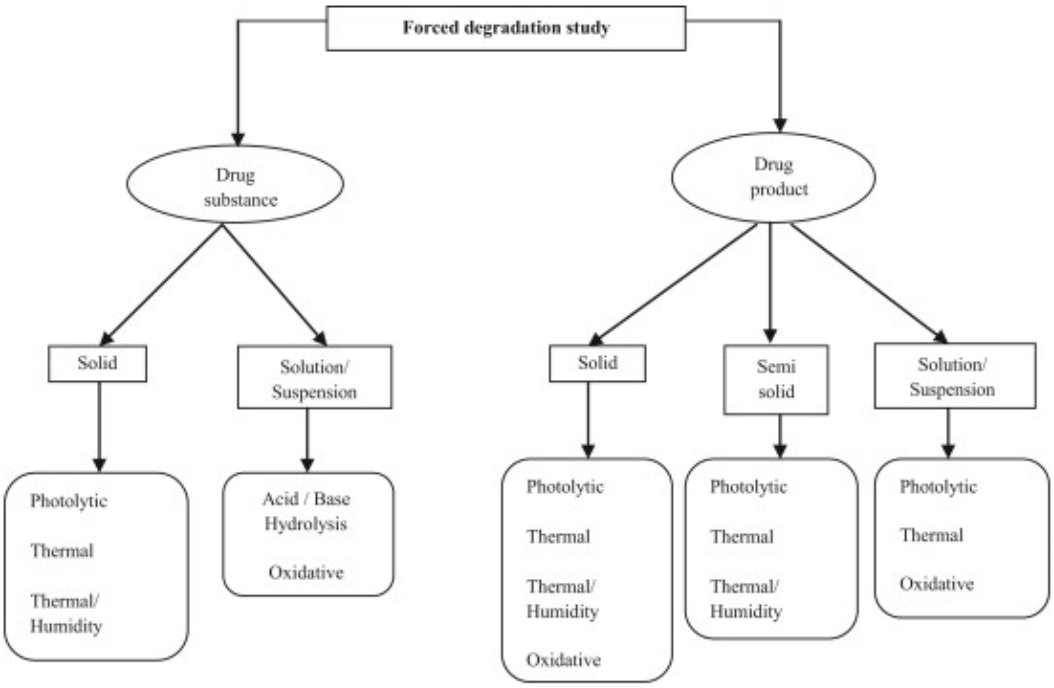
3.2 Typical stress test conditions for forced degradation studies

According to the evaluation of the currently regulatory relevant requirements forced degradation studies comprise a series of chemical and physical stress tests. Typical stress test conditions are:

- Heat (thermal stress with dry heat and / or wet heat)
- Photostability
- Hydrolytic stress - chemical stress (acid and base hydrolysis)
- Oxidation (oxidizing solution)

The applied stress conditions have to be defined case by case as the stress conditions depend on the decomposition of the drug substance or drug product and the final formulation e.g. during the normal manufacturing, storage and use conditions [31]. A general approach of use of degradation conditions for drug products of drug substances is provided in Figure 2.

Figure 2: General stress conditions used for drug substances and drug products for degradation studies [32]



3.2.1 Thermal stress tests - dry heat and wet heat

As indicated in the Figure 2 solid drug substances and drug products should be exposed to both thermal stress conditions dry and wet heat, while liquid drug products should be exposed to dry heat only.

According to ICH Q1A stress degradation studies should be conducted at more stringent conditions than introduced and recommended for accelerated experimental testing conditions [1].

Thermal degradation studies are normally conducted at 40˚C to 80˚C. The most widely accepted temperature is 70˚C at low and high humidity for 1-2 months [2]. High temperature (>80˚C) may not produce predictive degradation pathway predictive degradation pathway [33]. Wet heat can be applied the drug solution for several hours in order to see how much degradation occurs. It is recommended that the effect of temperature be studied in 10 ºC increments above that for routine accelerated testing, and humidity at 75% relative humidity or greater [1]. Studies may also be conducted at higher temperatures for a shorter period [34].

Humidity is seen as a key factor in establishing the potential degradants in the finished product and active pharmaceutical ingredient. Normally 90% humidity for duration of one week shall be recommended for the establishment of forced degradation samples [35]. Testing at several time points could provide information on the degradation rate and on primary and secondary degradation products [36]. In the event that the stress conditions produce minor or no degradation due to the stability of a drug molecule, one should ensure that the stress applied is in excess of the energy applied by accelerated conditions (40ºC for 6 months) before terminating the stress study [36].

3.2.1 Photolytic degradation

Photostability testing is accepted as an integral part of stress testing, especially when the drug substance or drug product are photosensitive. It needs to be confirmed that when a drug product or substance experiences an exposure to light this does not result in a not acceptable change. Some recommended conditions for photolytic degradation testing are described in the ICH guidelines According to ICH Q1B guideline [7] samples should be exposed to visible light providing:

- an overall illumination of a minimum of 1.2 million lux hours [7] and
- an integrated near ultraviolet energy of a minimum of 200watt hours/square meter with spectral distribution of 320-400nm to allow direct comparisons to be made between the drug substance and drug product [7].

Furthermore, the samples may be exposed side-by-side with a validated chemical actinometric system to ensure the specified light exposure is obtained, or for the appropriate duration of time when conditions have been monitored using calibrated radiometers/lux meters [7]. Looking into the literature the most commonly recommended maximum illumination is 6 million lux hours [37]. The most commonly accepted wavelength of light is in the range of 300–800 nm to cause the photolytic degradation [38, 39].

3.2.1 Hydrolytic degradation

Hydrolytic stress test is a common chemical degradation reaction of the analyte with water. Apart from water, hydrolysis reactions are normally performed over a wide range of pH by exposure of the sample to acidic or basic catalyzed stress conditions. The selection of the type for the stress testing - acid or base- depends on the stability of the sample. The stability of the analyte also defines the concentration. Hydrochloric acid (0.1–1 M) for acid hydrolysis and sodium hydroxide or potassium hydroxide (0.1–1 M) for base hydrolysis are the most common and suggested as suitable reagents for hydrolysis [40, 41]. The hydrolytic stress testing normally is conducted at room temperature with or without co-solvent and if no degradation appears, continues under higher temperature of 50°C to 70°C. Stress testing normally should not exceed more than 7 days. The degraded sample is then neutralized before injection using suitable acid, base or buffer, to avoid further decomposition. [42].

3.2.1 Oxidation degradation

Oxidative stress testing is one of the most conducted stress testing’s of drug degradation. When testing for oxidation, the common suggestion is to use hydrogen peroxide in the concentration range of 3% to 30%. But also other oxidizing agents can be used e.g. metal ions, oxygen, and radical initiators. In some drugs extensive degradation is seen when exposed to 3% of hydrogen peroxide for very short time period at room temperature. In other cases exposure to high concentration of hydrogen peroxide, even under extreme

condition does not cause any significant degradation. The behavior is on expected lines, as some drugs are in fact oxidisable, while there are others that are not [43].

In summary, which oxidizing agent should be taken, which concentration and conditions depends on the sample in question. When looking into the literature it seems to be reasonable to subject the samples to 0.1–3% hydrogen peroxide at room temperature (neutral pH) for seven days or up to a maximum 20% degradation. It is addressed that these conditions could potentially generate relevant degradation products [41, 42].

3.2.2 Current view on limits for forced degradation

How much degradation is sufficient to provide adequate and reliable data is a topic which is widely discussed. When the sample is overstressed this can lead to secondary degradation. Secondary degradation would not be formed in formal stability studies and would not support the purposeful stress testing [44]. Unfortunately the ICH guidelines are quite on how much degradation is required in forced degradation studies. When stressing too little some degradation pathways may not be identified and when the samples are stressed too much it can result in unrealistic degradation [45]. The extent of the stress applied in forced degradation studies should ensure formation of the desired amount (usually varies between 5 to 20%) of degradation [44]. Not always forced degradation studies result in product degradation. The degradation experiments can be stopped if no degradation is observed after drug substance or drug product has been exposed to a stress that exceeds accelerated stress conditions [36].

3.2.3 Analytical methods for identification of degradation products

Analytical procedures are used to assure that the drug product meets applicable standards of identity, strength, quality and purity during its expiration dating. CGMPs require stability indicating methods to monitor the drug product's stability profiles. When changes happen in drug product stability this can risk patient safety as degradation products can be formed. In order to monitor the possible changes to a product over time, the applied analytical method must be stability indicating.

According to the FDA guideline "Analytical Procedures and Methods Validation for Drugs and Biologics, Guidance for Industry" [46] a test is stability indicating if a procedure is a validated quantitative analytical procedure that can detect changes in a quality attribute(s)

of the drug substance and drug product during storage. Furthermore, the guideline outlines that to demonstrate specificity of a stability-indicating test, a combination of challenges should be performed [46].

The main objective of a stability indicating method is to monitor results during stability studies in order to guarantee safety, efficacy and quality. The degradation products have to be identified and quantified by an analytical method. If the degradation products are not identified by the selected analytical method, the method does not fit for the intended use. Analytical methods have to be validated to provide reliable data for regulatory submissions. Therefore method development and validation plays an important role towards having a stability indicating testing procedure and has a significant impact in the drug development process.

Forced degradation studies are used to facilitate the development of analytical methodology, to gain a better understanding of active pharmaceutical ingredient (API) and drug product (DP) stability, and to provide information about degradation pathways and degradation products.

High performance liquid chromatography (HPLC) is an integral analytical tool in assessing drug product stability and the most appropriate technique for developing/validating a stability indicating method. But also other stability indicating methods can be used e.g. TLC, electrophoresis, calorimetry, gel filtration etc. The premise is that the selected method is able to detect, separate, and quantify all observed degradation products that can be formed during manufacturing or storage. Furthermore should the method detect and quantify any impurities that may be introduced during synthesis.

Forced degradation is an integral part of the HPLC stability indicating method development. The introduced stress tests should produce representative degradation samples to test the selectivity of the method and to assess drug substance and drug product stability. Furthermore, the stress testing should provide information about possible degradation pathways and demonstrate the stability indicating power of the applied analytical procedures [47]. The quantitative determination of degradation is closely related to the evaluation of Limit of Detection (LoD) and Limit of Quantification (LoQ) of the method [47]. These limits should be based on the reporting, identification and qualification of degradation products, as stated in the ICH Q3B guideline [16]. The stereochemical stability of the drug, as well as physical and chemical properties important crystalline forms and the aspect of the mass balance are not directly related to conducting stress tests,

but should be taken into account [48, 49]. Stress tests should be performed with both: the active ingredient and the become formulation. [48]. Tests with the formulation provide information of interactions with excipients and the distinction between non-substance-related degradation products. Usually only one batch of the drug is stressed [1].

3.2.4 Time point for performing forced degradation studies

The time point when to initiate and perform forced degradation studies for drug products and drug substances is an important aspect. It seems that the most pharmaceutical companies conduct stress test studies on the drug substance and the drug product in the preclinical stage. Depending of the stage of the development the studies are repeated e.g. for the drug substance between the preclinical and registration and for the drug product between Phase I and registration [50].

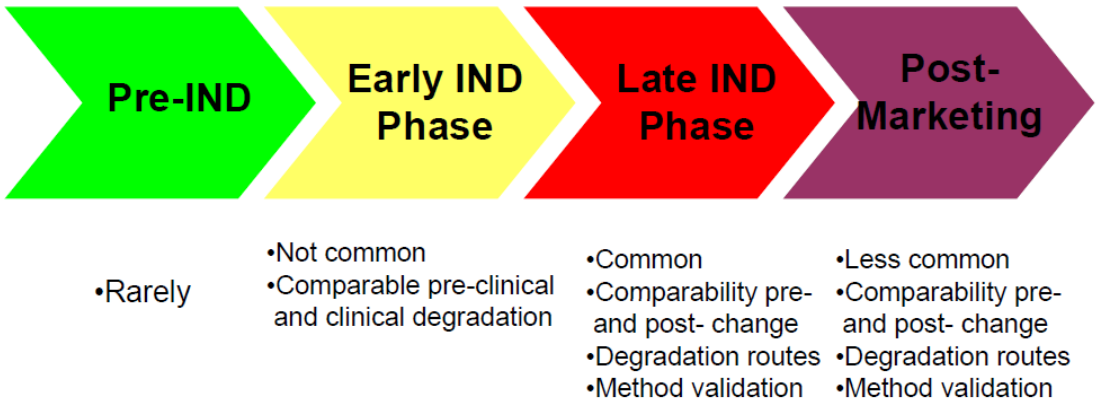
According to FDA the reporting of forced degradation study conditions or results is not required in phase I or II INDs, but encouraged to be performed [24]. The FDA guideline "Guidance for Industry INDs for Phase 2 and Phase 3 Studies Chemistry, Manufacturing, and Controls Information" outlines to perform stress testing in phase III of the regulatory submission process. These stress studies are conducted on a single batch. The results should be summarized and submitted in an annual report [24]. However, to start with the stress testing early in preclinical phase or phase I is highly recommended and should be performed on drug substance to obtain enough time for the identification of degradation products and structure elucidation. Furthermore this could help to optimize the applied stress conditions. A stress study at an early stage could also provide information for making improvements in the manufacturing process and in the selection of stability-indicating analytical procedures [24, 51].

As the information on the conducted forced degradation applies to multiple section of the CTD it should be presented as clearly as possible with hyperlinks and an understandable rationale to the other sections. Following CTD sections should be considered [51]:

- 3.2.S.7 Drug Substance Stability
- 3.2.P.8 Drug Product Stability
- 3.2.S.2.6 DS Manufacturing Process Development
- 3.2.S.3 Characterization
- 3.2.S.4.3 Validation of analytical procedures
- 3.2.P.2 Pharmaceutical Development

An evaluation on the current view on the timing of performing forced degradation studies based on the regulatory perspectives is given below in Figure 3 [51]:

Figure 3: Timing for performing forced degradation studies



In summary, stress tests during formulation studies (pre-IND Phase) are normally not performed, but could help to determine stability indicating quality attributes and degradation routes. During the pre-clinical phase stress testing is also not common, but could help to identify degradation products and potential of toxic components [52]. During the clinical development stress testing is common to conduct as a comparison of the pre-clinical and clinical quality provides a helpful input. After the drug product is on the market stress test studies are normally not performed, but in some cases e.g. when the manufacturing process changes stress testing could be very beneficial [52].

3.3 ANVISA – regulatory overview

3.3.1 Background and history on the ANVISA legal requirements regarding stability and forced degradation

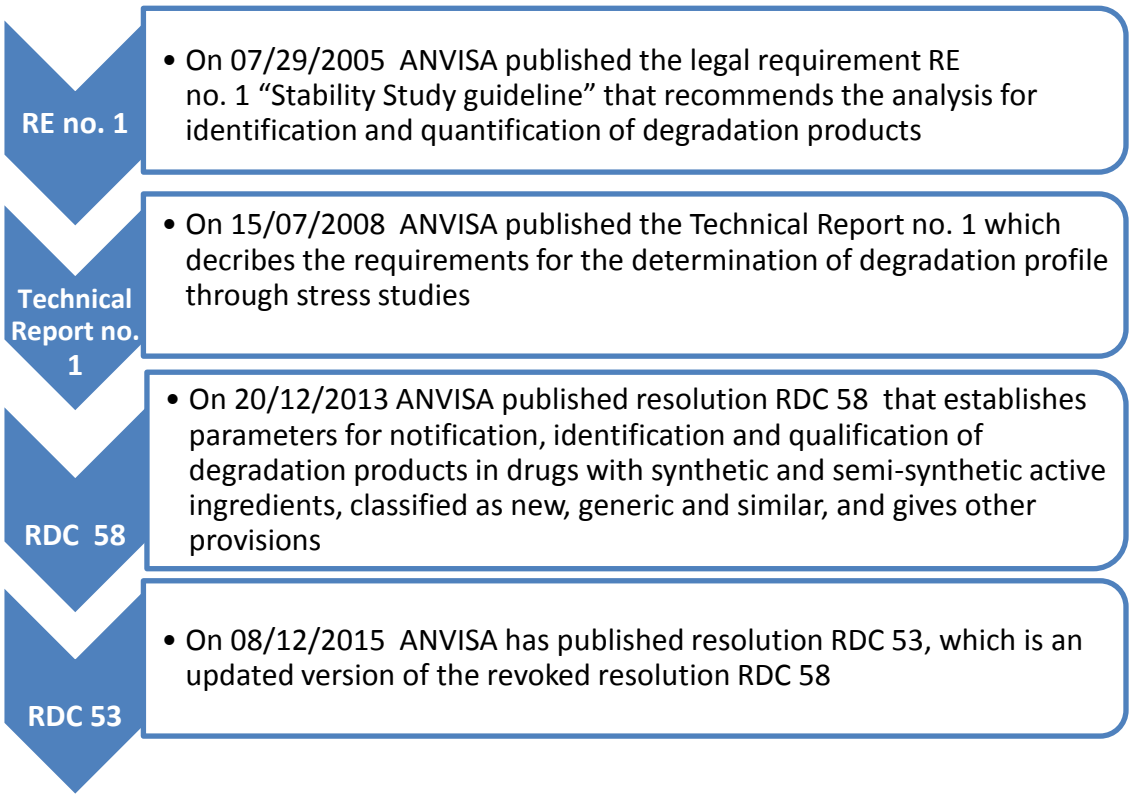
The National Health Surveillance Agency (ANVISA) was created by law 9782 in 1999 and is the governmental regulatory agency of Brazil, the largest country in south Amerika. The mission of ANVISA is “to protect and promote public health and to intervene in the risks caused by the production and use of products regulated by health surveillance. This mission must be carried out in coordination with states, municipalities and the Federal District, according to the Brazilian Unified Health System principles, in order to improve the quality of life of the population” [53].

In 2002 with resolution no. 50 and 2004 with resolution no. 398 ANVISA provided recommendations on long term stability testing conditions (30°C±2°C/70%±5%RH) and 30°C±2°C/65%±5%RH [54,55]. On July 29th 2005 ANVISA published a resolution the legal requirement RE No. 1 "Stability Study guideline". The resolution provides recommendations on long term conditions for Brazil (30°C±2°C/75%±5%RH) and on the analysis for identification and quantification of degradation products and corresponding analytical method [56].

On July 15th 2008 ANVISA published the Technical Report no. 1 [57]. This report was for the first time describing requirements for the determination of the degradation profile through stress studies. Technical Report no. 1 was cancelled and in December 20th, 2013 ANVISA published resolution RDC 58/2013.

Resolution RDC 58/2013 was establishing parameters for notification, identification and qualification of degradation products in drugs with synthetic and semi-synthetic active ingredients, classified as new, generic and similar. In December 2015 the resolution was replaced by the currently valid resolution RDC 53/2015. The development of the forced degradation legislation in Brazil by ANVISA is shown in the flow chart below (Figure 4).

Figure 4: Development on ANVISA'S forced degradation legislative



In order to understand the Agency (ANVISA) with respect how best to meet the new requirements of the resolution a regulatory guide on forced degradation was established. This regulatory guide (CP 68) covers following topics [4]:

- Realization of forced degradation studies
- Documentation to be sent to ANVISA with regard to the degradation profile
- Procedures for identification of degradation products
- Procedures for qualification of degradation products

3.3.2 General remarks to applicability and timelines of ANVISA's resolution RDC 53/2015

Resolution RDC 53/2015 establishes parameters for verifying degradation products in medications, for preparing the corresponding degradation profile and for reporting, identification and qualification of degradation products throughout the medication's shelf life [5]. Refer to article 14 of the resolution RDC 53/2015 for all registrations of new concentration inclusions or new dosage form inclusions the resolution comes into force on December 23rd 2015. For medicines which are already registered in Brazil the resolution includes timeliness with different dates for implementation of the requirements of this resolution [5]:

- Paragraph 1 of article 12 provides the timeliness for already registered medications as listed in Annex 1 (First level of therapeutic classes), of the resolution. For these medications resolution RDC 53/2015 shall become effective on December 31, 2017.
- Paragraph 2 of article 12 lists the timelines for already registered medications as listed in Annex II (Second level of therapeutic classes) of the resolution. For these medications resolution RDC 53/2015 shall be effective on December 31, 2019.
- Paragraph 3 of article 12 refers to other already registered medications which are not listed in Annex 1 and Annex 2. For these medications resolution RDC 53/2015 shall become effective on December 31, 2020.

However, according to article 12 ANVISA may request the start of specific monitoring of degradation products for the period prior to that described above, if there is any evidence of toxicity or loss of efficacy of the drugs described in paragraph 1, 2 or 3.

3.3.3 Comparison between resolution RDC 53/2015 and ICH guidelines and critical assessment

In following a comparison between resolution RDC 53/2015 and the information found in the ICH guidelines, mostly ICH Q3B, which has been identified as the corresponding guideline, is made. Furthermore, where identified and applicable, an attempt is made to provide a critical assessment of the similarities, differences, changes and new requirements.

Resolution RDC 53/2015: Refer to art. 2 the resolution RDC 53/2015 applies to "synthetic and semisynthetic active substances, classified as new, generic and similar" [5].

ICH: ICH Q6A guideline on specifications says in section 2.10 (Impact of Drug Substances on Drug Products Specifications [58]) that only impurities which are present in the new drug substance need to be monitored or specified in the new drug product, unless they are also degradation products and refers for further information to ICH Q3B guideline on "Impurities in New Drug Products". In the ICH Q3B guideline on "Impurities in New Products" only impurities of the drug substance or reaction products of the drug substance with an excipient and/or immediate container closure system (collectively referred to as "degradation products" in this guideline)" in new drug products are classified as degradation products [16].

Assessment: Compare to ANVISA's resolution RDC 53/2015 the ICH guidelines are more specific regarding the applicability e.g. are only impurities which are identified to be degradation products are addressed.

Resolution RDC 53/2015: Art. 2, Paragraph 1 of resolution RDC 53/ 2015 says that the resolution does not apply to: "biological/biotechnology products, excipients, peptides, oligonucleotides, radiopharmaceuticals, fermentation products and derivatives, herbal products, raw animal products, specific medications, vitamin-based medicines and/or minerals associated with each other or isolated, polyaminoacids, those with simplified notification, as well as products used in development of clinical trial stages". However article 2 paragraph 2 indicates that "for control purposes, degradation products of the products specified in paragraph 1 shall be adopted for specific tests, if any". Further it is indicated that "if faced with lack of specific tests, control must be guaranteed for those degradation products with significant toxicity or those that generate therapeutic inefficacy" [5].

ICH: The following types of products are not covered by ICH Q3B [16]: "biological/bio-technological products, peptides, oligonucleotides, radiopharmaceuticals, fermentation products and semi-synthetic products derived therefrom, herbal products, and crude products of animal or plant origin. Also excluded from this document are: (1) extraneous contaminants that should not occur in new drug products and are more appropriately addressed as good manufacturing practice (GMP) issues, (2) polymorphic forms, and (3) enantiomeric impurities". Further ICH Q3B does not cover impurities arising from excipients present in the new drug product or extracted or leached from the container closure system and is also not applicable to new drug products used during the clinical research stages of development [16].

Assessment: It seems that there is a conflict between paragraph 2 and paragraph 1. On the one hand for some categories of medicine the resolution RDC 53/2015 is not applicable; on the other hand to ensure control of product degradation in case of toxicity or therapeutic inefficiency, specific tests are requested. ANVISA attempts to ensure that pharmaceutical companies are responsible for the safety of their medicinal products and have a good knowledge of the relevant impurities from the drugs and know how to control them.

Resolution RDC 53/2015 and ICH including assessment: Article 3 of resolution RDC 53/2015 provides definitions which have been adopted for the purpose of this resolution. A comparison of the adopted definitions as included in resolution RDC 53/2015 article 3 and the definitions as included in the ICH guidelines is provided in Table 3.

Table 3: Adopted RDC 53/2015 versus ICH definitions [1, 5, 7, 15, 16, 58]

Term / Adopted resolution RDC 53/2015 definitions	Term / ICH definitions
Study of forced degradation Study that allows the generation of degradation products through exposure of active pharmaceutical ingredient and finished product to stress conditions, such as light, temperature, heat, humidity, acid basic and oxidation hydrolysis, among others.	**Stress testing (drug substance),** ICH Q1A Studies undertaken to elucidate the intrinsic stability of the drug substance. Such testing is part of the development strategy and is normally carried out under more severe conditions than those used for accelerated testing [1].

This study allows for the development of indicative methods for stability with adequate specificity and selectivity, as well for furnishing information about possible degradation means of a particular product	**Stress testing (drug product)** [ICH Q1A] Studies undertaken to assess the effect of severe conditions on the drug product. Such studies include photostability testing (see ICH Q1B) and specific testing on certain products, (e.g., metered dose inhalers, creams, emulsions, refrigerated aqueous liquid products).
Assessment: ICH does not include an exact definition for a study of forced degradation. Definitions are provided for the stress testing and do not address the same attributes. When looking into ANVISA's adopted definition for a study of forced degradation the determination of degradation products should be based on the forced degradation study. Refer to ICH Q3B the determination of degradation products should be based on the manufacture process, stability studies and laboratory studies [16].	
Impurity Any component contained in the pharmaceutical ingredient or finished product that is not the active pharmaceutical ingredient (API) nor the excipient(s)	**Impurity:** Any component of the new drug product that is not the drug substance or an excipient in the drug product [ICH Q3B]. 1) Any component of the new drug substance which is not the chemical entity defined as the new drug substance [ICH Q3A, ICH Q6A]. 2) Any component of the drug product which is not the chemical entity defined as the drug substance or an excipient in the drug product [ICH Q6A]
Assessment: The definitions regarding an impurity are comparable. ICH is a little bit more detailed and refers to "new" drug substances and drug products.	
Identification limit: Value above which a degradation product should have to identify its chemical structure	**Identification Threshold:** A limit above (>) which the impurity should be identified [ICH Q3A]

	A limit above (>) which a degradation product should be identified [ICH Q3B]
Notification limit Value above which a degradation product should be reported in stability studies	**Reporting Threshold:** A limit above (>) which an impurity should be qualified [ICH Q3A] A limit above (>) which a degradation product should be reported [ICH Q3B]
Qualification limit Value above which a degradation product should be qualified	**Qualification Threshold:** A limit above (>) which an impurity should be qualified. [ICH Q3B] A limit above (>) which a degradation product should be qualified [ICH Q3B]
Assessment: The definitions for the identification, reporting and qualification limits are similar. Nevertheless, the definitions from ANVISA are sometimes a little bit more specific and detailed.	
Degradation profile Description of the results and analytical activities used in detection, identification, structure elucidation and quantitative determination of degradation products present in the active pharmaceutical ingredient and medical product	**Degradation Profile**: A description of the degradation products observed in the drug substance or drug product [ICH Q3B].
Assessment: ICH only addresses the observed degradation products while resolution RDC 53/2015 provides a detailed summarized description on the degradation profile including analytical activities.	

Chromatographic peak purity of the API: Evidence that there is no interference of excipients, impurities and degradation products in the chromatographic peak of the API	---
Assessment: No definition available. ICHQ3B mentions the following "for the impurity tests, the impurity profiles should be compared. Peak purity tests may be useful to show that the analyte chromatographic peak is not attributable to more than one component (e.g., diode array, mass spectrometry)" [15].	
Degradation products Impurities resulting from chemical changes arising during manufacture or storage of the medication	**Degradation Product**: An impurity resulting from a chemical change in the drug substance brought about during manufacture and/or storage of the new drug product by the effect of, for example, light, temperature, pH, water, or by reaction with an excipient and/or the immediate container closure system [ICH Q3B].
Assessment: ICH provides a more detailed description the stress conditions and further effecting attributes. Moreover ICH provides further specified definitions for: • Unidentified Impurity/Identified Degradation Product [ICH Q3A, B] • Specified Impurity/Specified Degradation Product [ICH Q3A, B] • Unidentified Impurity/Unidentified Degradation Product [ICH Q3A, B] • Unspecified impurity/Unspecified Degradation Product [ICH Q3A, B]	
Qualification degradation products: Assessment of biological safety of an individual degradation product or a given degradation profile at a specific level	**Qualification:** The process of acquiring and evaluating data that establishes the biological safety of an individual degradation product or a given degradation profile at the level(s) specified ICH Q3B]
Assessment: The definitions regarding an impurity are comparable**.**	

Resolution RDC 53/2015: According to resolution RDC 53/2015 the study of forced degradation profile must meet the following requirements (art. 4) [5]: Conducting of the trial in a lot, laboratory, and pilot or industrial medication scale; and for comparison purposes, the study execution should also formulate placebo and isolated and associated active pharmaceutical inputs, in the case of associations in fixed dose.

1. The study of the forced degradation profile should be performed for all drug concentrations (Paragraph 1).
2. In the case of fixed-dose associations, a forced degradation studies with isolated and associated active pharmaceutical ingredients, and the formulation, should also be executed (Paragraph 2).

ICH: According to ICH Q1A the study is conducted on one batch [1].

Assessment: There is no ICH guidance regarding further details e.g. the batch origin, besides to conduct studies on one batch. Furthermore, there is no recommendation in the ICH guidelines to perform studies for comparison purposes. This means that pharmaceutical companies have to conduct forced degradation studies also with the placebo and the active ingredients alone, and the combined actives in the case of fixed-dose combinations. For example, in a case with more than three active ingredients the pharmaceutical companies will have to provide studies for the placebo, each of the active ingredients alone, for each of the three active ingredients combined. The testing of the active ingredient alone and with combination of some other active ingredients as well as the exclusion of some actives can be purposeful. It could help to demonstrate if a particular chromatographic peak is from a degradation product from the active ingredient or is related to an interaction of some active ingredients with each other. To test the placebo with each active substance seems also to be reasonable in order to differentiate which information is from the active and which from the placebo.

With regard to article 4, paragraph 1:

This requirement is contradictory to the bracketing approach described in the ICH guideline Q1D “Bracketing and Matrixing designs for stability testing of new drug substances and drug products” [59]: “The design of a stability schedule such that only samples on the extremes of certain design factors, e.g., strength, package size, are tested at all time points as in a full design. The design assumes that the stability of any intermediate levels is represented by the stability of the extremes tested. Where a range of strengths is to be tested, bracketing is applicable if the strengths are identical or very closely related in

composition (e.g., for a tablet range made with different compression weights of a similar basic granulation, or a capsule range made by filling different plug fill weights of the same basic composition into different size capsule shells). Bracketing can be applied to different container sizes or to different fills in the same container closure system". [59] It is not clear if a justified bracketing concept with a rationale confirming the overall validity of the results for all dose strengths in the protocol according to the ICH guideline Q1D could be proposed to ANVISA and would be accepted.

With regard to article 4, paragraph 2:

Even so no regulatory ICH guidance is available there is a trend to introduce drug products that contain more than one API to stress tests and assess for degradation produced by drug-drug and drug-excipient interactions [60]. Furthermore, it is emphasized that the compatibility of two drugs is not always addressed in the published literature of combination products [60]. However, operational challenges must be encountered when studies are performed with combination products, solutions of extremely stable products or use of organic solvents.

Resolution RDC 53/2015: Refer to article 5 a company must submit "studies subjecting the sample to the following forced degradation conditions: heat, moisture, acidic solution, basic solution, oxidizing solution, photolytic exposure and metallic ions. Additionally, the resolution emphasizes that for the case that the above conditions cannot be employed due to the inherent characteristics of the sample or if the conditions are not applicable, technical justification for non-use of any of these conditions must be made" [5].

ICH: ICHQ3B [15] says the following: "In particular, analytical procedures should be validated to demonstrate specificity for the specified and unspecified degradation products. As appropriate, this validation should include samples stored under relevant stress conditions: light, heat, humidity, acid/base hydrolysis, and oxidation.

Assessment: ANVISA asks to establish a new stress condition - the forced degradation by metal ions without providing further information. None of the ICH guidelines have included recommendations or requirements to perform a test with metal ions. It is well established that metal ions catalyze oxidative reactions. For example, Cu is an extremely effective catalyst able to accelerate the oxidative reaction up to several thousand times. When searching the literature it is reported that different reactions happen when metal ions are present. This leads to a suggestion that stress studies with metal ions should be part of

the forced degradation studies [41]. According to the single paragraph of the same article if the described conditions cannot be employed due to the inherent characteristics of the sample or is not applicable, the technical justification for non-use of any of these conditions must be made. The question arises what should such a technical justification include in order providing an acceptable justification to the agency. Would a confirmation of absence of metal ions e.g. not metal equipment's or packaging materials containing metal ions are used, be sufficient?

Resolution RDC 53/2015: In article 6 it is indicated that forced degradation studies should promote degradation to the extent which is sufficient to allow evaluation of formation of degradation products. The tests should promote degradation greater than 10% (ten percent) and less than that which would lead to complete degradation of the sample, thereby compromising the test. In tests where degradation is less than 10% (ten percent), the company must provide a technical justification. The achieved results of the tests are supposed to support the development and validation of analysis methods of the products formed by degradations and critical analysis of the medication degradation profile [5].

ICH: None of the ICH guidelines specifies the exact value of degradation during the study.

Assessment: The question of how much degradation is sufficient to meet the objectives of stress studies is widely discussed, especially with respect to conventional therapeutics. If too much stress is applied then unrealistic degradation products may be observed and the resulting analytical method may be unsuitable for detecting actual degradation products formed during stability testing. Thus, the actual conditions need to be chosen carefully so that the amount of degradation of the drug substance produced during forced degradation is neither too excessive nor too little. [36]. Forced degradation experiments do not necessarily result in product decomposition. The study can be stopped if no degradation is observed after drug sample or drug product has been exposed to a stress that exceeds conditions of accelerated stability protocol [61]. ANVISA asks for an extent of degradation over 10%. This raises the question for an appropriate level of degradation. What product parameters characteristics should be taken into account and are important to assess? Some products are very stable and do not degrade easily. Incases test do not exceed 10% of degradation ANVISA asks for a technical justification. Unfortunately, there is no information regarding technical justification included. It is not clear if for example a justification based on literature confirming that the product in question is stable and does not form degradation products under extreme stress conditions, would be acceptable. Or should this justification

be accompanied by some additional testing to confirm that the product in question behaves as reported in the literature?

Resolution RDC 53/2015: In article 7 the critical analyses of the achieved degradation profile is addressed: "Verification of the peak chromatographic purity of the active pharmaceutical ingredient in the medication; and evaluation of the factors that may interfere in any way in the stability of the medication" [5].

ICH: No Information was identified in the ICH guidelines.

Assessment: "Peak purity is an analysis of absorbance spectra across the peak to determine if they are all similar if there are differences. If there are differences, it implies there are two or more compounds eluting in that chromatographic peak each being spectrally different" [62]. ANVISA requests the companies to ensure the purity of the peak. To ensure peak purity different considerations have to be taken into account and a verification of peak purity is not feasible to provide for all methods. E.g. when it is not possible to verify the peak purity with photodiode detector, one of the common methods for demonstrating that the peak corresponds to a single component, the company is challenged to use other procedures to ensure that there is no co-elution.

Resolution RDC 53/2015: Art. 8 [5] lists the tests and the results of the forced degradation testing should be redone and resubmitted when requested:

- Changes or additions to the synthesis route of the active pharmaceutical ingredient; or qualitative and quantitative changes in the composition of the finished product.
- When there is more than one active pharmaceutical ingredient manufacturer, the results of forced degradation should be assessed for each manufacturer.
- In the case of quantitative changes in the excipient, may be sent to study the degradation profile and technical justification with rationale for use of forced degradation study ever conducted with the former formulation without the need for conducting a new study of forced degradation. The technical justification must demonstrate the inability to form new degradation products.

ICH: None of the ICH guidelines was identified to request performing of forced degradation re-testing and resubmission in case of changes as described above.

Assessment: According to the current understanding the evaluation of post-registration changes e.g. manufacturing changes is based on stability studies. This requirement will have a big impact for the pharmaceutical companies when changing API synthesis route, manufacturing process of the finished product and manufacturer of API. Since suggested changes are going along with an improvement for the product a simplified "fast track" procedure would be desirable to get the improved changes implemented quickly. Furthermore, it seems that the requirement to test APIs from each manufacturer a bit too strict. As the chemical structure from the active pharmaceutical ingredient remains the same it could be assumed that also the degradation profile would remain the same.

Resolution RDC 53/2015: Article 9 provides thresholds for degradation products and information for identification and qualification of the degradation product(s) which during the drug stability study should be evaluated [5]. The thresholds as stated in resolution RDC 53/2015 [5] are provided in Table 4.

Table 4: Thresholds for degradation products according to resolution RDC 53/ 2015

	Maximum Daily Dose 1 a	**Limits 2 b**
Notification Limits	≤ 1 g	0.1%
	> 1 g	0.05%
Identification Limits	< 1 mg	1.0% or 5 µg ATD [c], whichever is less
	1 mg – 10 mg	0.5% or 20 µg ATD [c], whichever is less
	> 10 mg – 2 g	0.2% or 2 mg ATD [c], whichever is less
	> 2 g	0.10%
Qualification Limits	< 10 mg	1.0% or 5 µg ATD [c], whichever is less
	10 mg – 100 mg	0.5% or 200 µg ATD [c], whichever is less
	> 100 mg – 2 g	0.2% or 3 mg ATD [c], whichever is less
	> 2 g	0.15%

a Maximum amount of the active pharmaceutical ingredient administered per day.
b Limits of the degradation products are expressed as the percentage of the active pharmaceutical ingredient or as the total daily administration (TDA) of a degradation product
c Average Daily Dosage

ICH: The thresholds derived from ICH Q2B [16] are shown in Table 5.

Table 5: Thresholds for degradation products according to ICH Q3B

Attachment 1: Thresholds for Degradation Products in New Drug Products

Reporting Thresholds

Maximum Daily Dose[1]	Threshold[2,3]
≤ 1 g	0.1%
> 1 g	0.05%

Identification Thresholds

Maximum Daily Dose[1]	Threshold[2,3]
< 1 mg	1.0% or 5 µg TDI, whichever is lower
1 mg - 10 mg	0.5% or 20 µg TDI, whichever is lower
>10 mg - 2 g	0.2% or 2 mg TDI, whichever is lower
> 2 g	0.10%

Qualification Thresholds

Maximum Daily Dose[1]	Threshold[2,3]
< 10 mg	1.0% or 50 µg TDI, whichever is lower
10 mg - 100 mg	0.5% or 200 µg TDI, whichever is lower
>100 mg - 2 g	0.2% or 3 mg TDI, whichever is lower
> 2 g	0.15%

Notes on Attachment 1

1 The amount of drug substance administered per day

2 Thresholds for degradation products are expressed either as a percentage of the drug substance or as total daily intake (TDI) of the degradation product. Lower thresholds can be appropriate if the degradation product is unusually toxic.

3 Higher thresholds should be scientifically justified.

Assessment: The thresholds as introduces by ANVISA are comparable to the thresholds included in the ICH Q2B and comply with each other. However, as indicated by ANVISA after the molecule identification, if there is a structural alert for the presence of genotoxic moiety, the product safety profile must be established immediately. In addition, ANVISA will consider the relevance of the defined safety profile and may not accept the specification given by the company [5]. When looking into ANVISA'S degradation profile guide the toxicological tests used for identification of impurities are following the internationally accepted guidelines.

Resolution RDC 53/2015: According to article 11 the acceptance limits for each product degradation individually and the total limit of degradation products should be included in the specifications of the release of medication and stability studies.

Furthermore, the degradation product that exceeds the notification threshold should be included in the release specifications for the medication and the stability study [5].

ICH: ICH Q2B [15] outlines similar requirements by indicating the new drug product specification should include, where applicable, the following list of degradation products

- Each specified identified degradation product
- Each specified unidentified degradation product
- Any unspecified degradation product with an acceptance criterion of not more than (≤) the identification threshold
- Total degradation products

Assessment: The requirements on this topic are comparable between resolution RDC 53/2015 and ICH guidelines.

Resolution RDC 53/2015: According to article 15 a specific protocol with relevant documentation is required.

ICH: None of the ICH guidelines were identified that specifically addressed to provide a product degradation profile protocol to the regulatory agencies. When looking into ICH Q1A the most experimental designs are left to the applicant's discretion [1].

Assessment: Although, none of the regulatory agencies request a product degradation profile protocol it would be helpful to have this document in place. However, it has to be kept flexible as forced degradation is a topic for research and development. Some guidance which information with respect to the performed forced degradation studies should be included in the product degradation profile protocol is included in ANVISA'S degradation guide. A discussion on the content is provided in section 5 Outlook.

4. Discussion

As already assessed in the previous chapters there are still quite a few open points and inquiries with a need for further discussions, clarification and assessment.

It should be kept in mind that forced degradation studies do not simulate a real storage or transport condition. Therefore the chosen stress conditions used to exceed the stability profile of the drug product. The question rises if the results derived from the forced degradation studies could lead to misleading stability interpretation.

Following points were assessed to be critical:

- to present a accepted technical justification if 10% degradation of the active ingredient(s), is not achieved during the forced degradation studies

- to include, where applicable, degradation products in the release specification
- to establish new degradation conditions with use of metal ions beyond relevant stress conditions: light, heat, humidity, acid/base hydrolysis and oxidation
- to consider the critical analysis of the degradation profile: confirmation of chromatographic purity of the peak of the active pharmaceutical ingredient in the drug product
- to provide forced degradation studies for the purpose of comparison the execution of the study must also be done with formulation, with placebo and in active pharmaceutical ingredient(s), alone and in combination in case of fixed dose
- to provide forced degradation studies forced degradation studies for all drug concentrations
- to provide data and establishing retesting of forced degradation in case of: modification/alterations or inclusions in the APIs route of synthesis or quantitative and qualitative changes of excipient in the composition of the finished product

5. Outlook

With coming into force of resolution RDC 53/2015 ANVISA requests the pharmaceutical companies to provide a protocol on the degradation profile based on the performed forced degradation studies. Article 15 states for the cases referred to in paragraphs 1, 2 and 3 of the article 14 the compliance with this resolution shall be performed through a specific protocol with relevant documentation and paragraph 1 of the same article indicates that absence of a specific protocol at the time of effect of the resolution, allows ANVISA to determine the production suspension until meeting compliance or can cancel the registration of the product [5]. In order to fulfill the new regulatory requirements of ANVISA it would be of great advantage to have a best practice for performing forced degradation studies and for a protocol in place which provides guidance how to record and communicate the achieved results and the degradation profile to the agency. However, such a best practice or a protocol should allow a lot of flexibility to enable further development and changes as forded degradation is a topic for further development. According to the regulatory guide the objectives of studies for obtaining the forced degradation profile are the following [4]:

- to obtain the qualitative degradation profile of the drug or medicine
- to prove that a proposed method is stability indicating
- to detect conditions to which the drug is particularly sensitive in order to alert the Quality Assurance System of the company for particular care to be taken in the development, production, handling, and storage of this product
- to identify specific markers for a particular product degradation and, where possible, to facilitate possible deviations

The regulatory guide amplifies that the degradation profile consists of two parts: the critical phase and the experimental phase. The critical part needs to be performed before starting with the experimental part. The critical part involves literature research including manufacturers DMF and official compendial monographs. According to the guide [4] the purpose is to gather information of e.g. functional group chemistry, the potential interactions with the excipients or potentially possible degradation products with alerts for toxicity or genotoxicity [4]. In following an attempt is made to discuss a potential content of a protocol for the experimental part. The proposed protocol provides a brief description of the parameters to be performed for stress testing (forced degradation) during the laboratory testing in order to establish a degradation profile for the product in questions according ANVISA' requirements. The protocol for a degradation profile protocol is discussed based on the understanding of the resolution RDC 53/2015 and taking into account ANVISA's regulatory guide and has more general character. Not all single aspects of the resolution can be discussed and covered.

5.1 Degradation profile protocol

5.1.1 Purpose of degradation study

In this section the purpose of the degradation study should be described e.g. the purpose of the study is to investigate and evaluate the degradation profile of drug product, API and placebo to drug product in order to gain to gain supportive information for the generation of the degradation profile. Furthermore, the applied stress conditions (thermal [wet and dry heat], photolytic, oxidative, hydrolytic and metals ions) should be described in order to investigate the formation of potential degradation products and to show the selectivity of the test method towards potential degradation products of the drug substance under stress

conditions and to demonstrate the method is stability indicating. Additional information on the susceptibility of the drug product to agents potentially causing degradation should also be provided. In case any condition cannot be employed due to the characteristics inherent to the sample, an officially recognized justification (e.g. Pharmacopoeia) for omitting the condition needs to be provided. If there is more than one API manufacturer involved, this should be described and studies need to be described for each manufacturer. Information that reference samples (unstressed samples) will be tested as well should be included.

5.1.2 Information on the structural formula

This section should include information on the structural and molecular formula. Furthermore the known impurities should be included and discussed taking into account the relative response factors (RRF values) and relative retention times (RRT). This information can be derived from an official compendial monographs, scientific literature or information from the manufacturer. A tabulated presentation of the information as shown in Table 6 would be beneficial.

Table 6: Structural and molecular formula, chemical name of drug substance/ characterized impurities

Chemical name (e.g. Ph. Eur.)	Molecular formula	Structural formula
Drug substance 1,2 etc.		
Impurities 1,2 etc.		

5.1.3 Analytical procedure

A description of the used analytical method including a justification regarding the suitability of the method to be able to detect degradation products should be included. A reference to the sited suitable testing procedure should be provided.

5.1.4 Overview of the performed Studies

An overview of the performed studies should be provided. According to article 4 following scenarios are possible [5]:

- API 1; API 1 (1. manufacturer) / API 2; API 2 (2. manufacturer). etc.

- API 1 + API 2
- Drug product strength 1/ drug product strength 2 / drug product strength 3, etc.
- Placebo

Information on the chosen stress conditions should be included taking into account an achievement of reduction of 10% to 30% in peak area of the active (s) ingredient (s). If reduction can't be achieved in reasonable conditions or time period described (more rigorous conditions can be applied than described), a justification of the stability of the active substance should be presented. Additionally, applied stress conditions should be listed together with the evaluated stress conditions as well as the selected test parameters. The selection on appropriate conditions and parameters depends on the product in question. A possible approach is provided below in Table 7.

Table 7: Stress conditions

Stress condition	Conditions	Duration
Thermal stress (dry heat)	80°C	2 weeks
Humidity stress (moist heat)	80°C/100% RH	2 weeks
Photo stress	ICH Q1B conditions: 1.2 kLxh 200 Wh/m^2 (UV)	2 weeks
Chemical stress:		
Acid	0.1 M HCL/pH=1/80°C	2 days
Alkaline	0.1 M NaOH/pH=13/80°C	2 days
Oxidation	0.3% H_2O_2 /RT	10 days
Metal ions	0.05 M Fe(II) sulfate /RT 0.05 M Cu(II) sulfate/RT	2 week 2 week

5.1.5 Results of the studies

According to CP 68 [4] the degradation profile should include testing of those attributes of the FPP that are susceptible to change during storage and are likely to influence quality, safety and/or efficacy. For instance, in case of tablets test results of following attributes e.g. appearance, hardness, friability, moisture content, dissolution time, degradants and assay should be included. Furthermore, the test results for each study e.g. API, drug

product, placebo and each test condition (thermal stress [dry and wet heat], photolytic, oxidative, hydrolytic and metals ions stress) should be included.

In Table 8 a proposed description for the metal ion stress is included. API 1, API 2, API 1 + API 2, drug product and placebo to drug product were dissolved in a solution of e.g. 0.05 M Fe^{2+} or Cu^{2+} solution and stored in closed glass containers at e.g. 70°C for e.g. 2 weeks to achieve a reduction of 10% to 30% in peak area of the active ingredient.

A tabulated overview of the assay and degradation profile for API 1, API 2, API 1 + API 2, drug product and placebo to drug product at RT with 0.05 M Fe^{2+} or Cu^{2+} as exemplarily shown should be provided.

Table 8: Assay and degradation profile at 70°C in Fe^{2+} and Cu^{2+} solution

Test Substance	Peak no.	Retention time (min)	Peak Area
Injection peak	1		
Solvent	2		
API1	3		
API 2	4		
API 1 + API 2	5		
Drug product	6		
Placebo	7		

Additionally, the chromatograms of API 1, API 2, API 1 + API 2, drug product and placebo to drug product at tested condition should be included. Reference samples for comparison purposes should be provided and analyzed without being subjected to stress conditions.

5.1.6 Evaluation and conclusion of the degradation studies

It should be evaluated if under all investigated stress conditions the observed unknown degradation products are sufficiently separated from the drug substance peak or peaks of identified organic impurities. In addition, it has to be shown that the peak of the investigated drug substance did not show any sign of co-elution of degradation products when investigated with e.g. PDA (photodiode detector array) and drug substance in all investigated stress test conditions. An assessment regarding mass balance considering if the results comply with relative standard deviation found in precision of method validation should be provided. Furthermore, a critical evaluation of the product in question for each single stress conditions should be included. For example is following evaluation for the

stress condition with catalytic metal ions possible: The drug product shows good stability with regard to catalytic/metal ions stress. Only under severe stress test conditions with Fe^{+2}/Cu^{+2} a significant degradation was observed. The observed unknown degradation products are sufficiently separated from the drug substance peak.

The conclusion should include a summary concerning the results obtained in the stress test (potential degradation profile) and a critical assessment regarding a potential impact of the forced degradation studies study results e.g. if degradation impurities have to be included into the specification of the product. Finally, the suitability of the method for detection of degradation products should be confirmed as well as that the method is stability indicating (all peaks were sufficiently separated from the drug substance peak and show no sign of co-elution).

6. Conclusions

It is uncontroversial that stability is a critical quality attribute of the drug substance and the drug product and that stability profiles need to be established for drug product to assure safety, efficacy and quality. Well thought out forced degradation studies can support the establishment of products stability profiles. In the past years, many studies on forced degradation have been performed and reported in the literature. However, as only minor regulatory guidance is available, many of the studies provide insufficient, sometimes even contradictory information and results.

In one of the stress testing benchmarking studies 20 pharmaceutical companies provided information regarding conduction of stress tests. The results showed a significant variety between the pharmaceutical companies. For example, a degradation range of 5- 20% could be observed before the companies stopped the testing studies. Also the stress conditions e.g. temperatures, pH conditions, and the duration of studies did significantly vary [63].

Pharmaceutical companies aim to provide suitable information on forced degradation when compiling the information as a part of the high quality dossier for submission to the regulatory agencies. But with the current regulatory situation for forced degradation it is getting more and more challenging to design adequate forced degradation studies and provide high quality data. On the one hand the companies have to deal with only minor regulatory guidance for the ICH countries and on the other hand since December 2015 to face strict requirements from the Brazilian legislation for the product on the Brazilian market. The International Conference of Harmonization (ICH) has achieved a great deal of harmonization also with regard to stability, but there is still need for further improvement and harmonization, especially with regard to forced degradation. Now, with coming into force of ANVISA's new resolution RDC 53/2015 and the new requirements on forced degradation have to be implemented by the companies for the Brazilian market, in the author's opinion it would be of great achievement if ICH reacts on the current unsatisfactory regulatory situation. The general aim should be to achieve harmonization on the current standards for forced degradation. Helpful would be to have an ICH guideline on forced degradation in place containing specific requirements and recommendations on performing purposeful forced degradation studies in order to enable the pharmaceutical companies to create high quality data without wasting time, capacities and resources.

In the next years it will be necessary to monitor the implementation of Resolution RDC 53/2015 and it would be advisable to react with other new approaches and proposals if it turns out that some of the established requirements have little or no benefit.

As the forced degradation is a developing field and still a matter for further research the goal should be to work together on further improvement and harmonization of standards where only possible. At the end the focus of all pharmaceutical companies is to generate and present high quality forced degradation data in order to assure safety, efficacy and quality of the product in question.

7. Summary

This master thesis provides a general overview on the topic forced degradation, the purpose of performing forced degradation studies, the available regulatory guidance as provided by ICH, EMA, FDA and WHO and the current understanding and approach driven by the pharmaceutical companies. In addition, the new legislation for reporting, identification and qualification of degradation products in medications established by ANVISA, the National Health Surveillance Agency of Brazil with resolution RDC 53 from December 2015, is introduced.

A comparison between the new Brazilian legislation and the available regulatory standards is made, following by a critical assessment on the differences and the discussion of the challenges and critical points for the pharmaceutical companies. Concluding a degradation profile protocol of a drug product, requested by ANVISA for future submissions, is discussed. In summary, the introduction of the new Brazilian legislation regarding forced degradation includes several changes and challenges for the pharmaceutical companies, but also chances and opportunities.

As discussed in the thesis, the currently available regulatory guidance regarding forced degradation is incomplete and very general. Therefore, the provision of new regulatory requirements and guidance for the pharmaceutical companies was an urgent need. Although, there are still quite a lot of open questions, with resolution RDC 53/ 2015 for the first time a national regulatory agency provided regulatory binding requirements and guidance on the forced degradation topic.

The pharmaceutical companies should use the opportunities and advantages from the new resolution RDC 53/2015 and implement where possible and reasonable new standards globally. This approach would help to conduct purposeful forced degradation studies resulting in generation of high quality data and provide a voluble contribution with relation to better quality, safety and efficacy of medicinal products.

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Hiermit erkläre ich an Eides statt, die Arbeit selbständig verfasst und keine anderen als die angegebenen Hilfsmittel verwendet zu haben.

Bonn, March 2016

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Helene Janzen