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Overview on Development and Validation of Force Degradation Studies with Stability Indicating Methods

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This article provides an overview on Development and Validation of Force degradation study with Stability Indicating Methods (SIMs) for drug substances. Different stress conditions (hydrolysis, oxidation, thermal, and photolytic conditions) are applied to drugs compounds during a process known as forced degradation, and as a result, various degradation products are created. The major purpose of these investigations is to assess a molecule's stability under accelerated settings. It is well recognized that the stability of molecules affects the regulatory documentation process, the choice of appropriate storage and packaging conditions, and the choice of formulation. The deficiencies of reported methods in terms of regulatory requirements are highlighted. This article is to provide appropriate International conference of harmonization (ICH) criteria for force degradation study and to discuss the methodical process for creating verified SIAMs. The aspects of Mass balance in SIMs are discussed and technique used in SIMs were highlighted. Recent advance in stability indicating methods include characterization of degrdant sample and in-vitro toxicity prediction are addressed. Background of force degradation study with stability indicating methods with respect to international author and national author are discussed.

Keywords: Force degradation study; Mass balance; Stability Indicating Methods; toxicity study.

The ability of the pharmaceutical dosage form to maintain the physical, chemical, therapeutic, or microbiological properties throughout storage and patient usage is referred to as drug stability. Due to its impact on the security and effectiveness of the drug product, drug molecule stability is a major concern.[1] Consequently, it is important to understand a drug's purity profile and how it behaves in different environmental conditions. The choice of an appropriate formulation and package, in addition the provision of appropriate storage conditions and medication shelf life, are all made easier with knowledge of the drug's molecular structure, which is crucial for regulatory documentation.[2]

Studies undergoing forced deterioration are often referred to as stress examinations, stress studies, studies on stress decomposition, and studies undergoing forced decomposition. The International Conference on Harmonization (ICH) guideline entitled "Stability Testing of New Drug Substances and Products" (Q1A) was

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introduced in 1993, and although these studies have long been used in industry, they now have a formal regulatory obligation. Because it has an impact on the safety and effectiveness of the drug product, the chemical stability of drug molecules is crucial.[3] According to the FDA and ICH recommendations, stability study data is necessary to understand how a drug substance's and drug product's quality alters over time under the effect of different various environmental conditions like temperature, PH, light, etc. The concentration of the active pharmaceutical ingredient (API) in a drug product decreases as it is subjected to different degradation circumstances. This concentration decline is estimated using a stability indicating method (SIM), an analytical approach. The creation of a reliable stability indicating method offers a reliable foundation for pre-formulation research, stability investigations, and the maintenance of safe drug substance and drug product during storage conditions.[4] survey of the literature finds that the term "stability-indicating" was used to describe many different techniques that were described during the course of the last three to four decades. Most documented approaches, nevertheless, don't quite measure up to the standards set by the law at the moment. As a result, the goal of this article is to provide appropriate ICH criteria for force degradation research and to discuss the methodical process for creating verified SIAMs. Additionally, the employment of several SIAM techniques and other crucial topics, like the degree of separation of degradation products and the establishment of mass balance, are discussed.

Regulatory status of stability-indicating assays

In actuality, more nations than just the EU, Japan, and the US use the ICH standards despite their incorporation into national legislation in just three regions. The de facto power of regulation is carried by these recommendations because they mirror the present inspectional tendencies. Following table 1 provide ICH guidelines:

The manufacturer is required to present a stability-indicating profile that ensures the identification of changes in the product's identity, purity, and potency because there is no single test or parameter that profiles the stability characteristics of such goods. Unfortunately, there is no precise definition of a stability-indicating approach in any of the ICH guidelines. However, the United States-Food and Drug Administration (FDA) provides detailed descriptions of stability-indicating methods. According to a 1987 guideline, In order to accurately measure the active ingredient content, stability-indicating methods were defined as "quantitative analytical methods based on the characteristic structural, chemical, or biological properties of each active ingredient of a drug product and that will distinguish each active ingredient from its degradation products. As stated in the draught guideline from 1998, "validated quantitative analytical methods" are "validated quantitative analytical methods that can detect the changes with time in the chemical, physical, or microbiological properties of the drug substance and drug product, and that are specific so that the contents of active ingredient, degradation products, and other components of interest can be accurately measured without interference."[5-13]

Background of force degradation studies with stability-indicating assay

Some International authors like John W. Dolan *et al* and Dan W. Reynolds *et al*, Karen M. Alsante *et al*, Silke Klick *et al,* George Ngwa *et al* and Christine Nowak *et al* Overview on force degradation studies with stability indicating assay methods. They have discuss various point related to force degradation studies and stability indicating methods. The following figure 1 show the highlight of studies done by international authors.[14-16]

The National author like Saranjit Singh *et al,* Monika Bakshi *et al*, Renu Sehrawat *et al*, Blessy M *et al*, Manvi Hasija *et al*, Trivikram Rawat*et al*, Manish Kumar Sharma *et al*, Panchumarthy Ravisankar*et al,* Suvarna Sopanrao Thorat *et al,* **and** Sowmyalakshmi Venkataraman discuss various point related to development and validation of stability indicating methods. The highlights of individual authors given in following figure 2.[19-24]

Literature report on stability indicating methods meet current requirements

A review of Development of validated stability indicating assay methods- Critical review was published in 2002. There are very few approaches that are fully fixed into stability indicating ways, according to numerous literature publications from 1976 to 2000. Thus, very few studies that expose the medication to various stress conditions and make an effort to differentiate the drug from degradation products and the latter

among themselves are actually stability-indicating investigations. We evaluate literature reports on stability indicating approaches from the years 2001 to 2020 in this review paper. In some paper it has been demonstrated that drugs can be purified from known synthetic contaminants and/or possible degradation products without being stressed in any way (Table 2).It has also been reported that drugs have broken down after being subjected to one (Table 3), two (Table 4), three (Table 5), four, or more (Table 6) stress situations. Depending on the nature of the active medicinal components, various analytical methods were employed. In several cases, stress experiments have been performed on medication formulations or even drug combinations rather than the actual drug component to create stability-indicating behaviour (Table 7). It might be important to note that the examples given in Tables 1-6 are simply meant to be illustrative and do not necessarily reflect a complete list of all literature reports.

Techniques used in stability indicating methods

Techniques including titration, spectrophotometry, and chromatography have been often used to analyse stability materials. In following figure 3 shows instrument and apparatus used in analysis of sample. Chromatographic technique like HPLC (High performance liquid

Table 1. ICH guidelines

Q1A	Stability Testing of New Drug Substances and Products
Q1B	Photo stability testing of new drug substances and products
Q1C	Stability testing of new dosage forms.
Q1D	Bracketing and matrixing design for stability testing of drug substances and products
Q1E	Evaluation of stability data
Q1F	Stability data package for registration applications in climatic zone III and IV
Q2(R1)	entitled 'Validation of Analytical Procedures: Text and Methodology'
Q3A(R2)	'Impurities in New Drug Substances' under section 3. Rationale for the Reporting and Control of Impurities, subsection 3.1 Organic Impurities
Q3B	entitled 'Impurities in New Drug Products' under Section 3, Analytical Procedures, also has a mention of stress conditions
Q6A	Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances
Q5C	on Stability Testing of Biotechnological/Biological product
Q5E	entitled 'Comparability of Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process'
Q7A	ICH guideline on Good Manufacturing Practices for Active Pharmaceutical Ingredients (Q7A), which is under adoption by WHO,

Table 2. Report of 'Stability Indicating' methods Where No stress testing done. [25-34]

Drug	Methodology	References
Sildenafil citrate	RP-LC	25
Lisinopril	HPLC	26
Miconazole nitrate	HPLC	27
Cefuroxime Axetil	RP-HPLC	28
Captopril	HPLC	29
Domperidone (DP), Methylparaben (MP) and Propylparaben	HPLC	30
Quetiapine	HPLC	31
Clopidogrel	Micellar Liquid	32
Paracetamol	TLC Densitometry	33
Paracetamol and Chlorzoxazone	TLC Densitometry	34

chromatography), HPTLC (High performance thin layer chromatography) and UPLC (Ultra performance liquid chromatography) used for separation of multiple components during analysis of stability samples. Most recent publication used HPLC and HPTLC method for analysis of

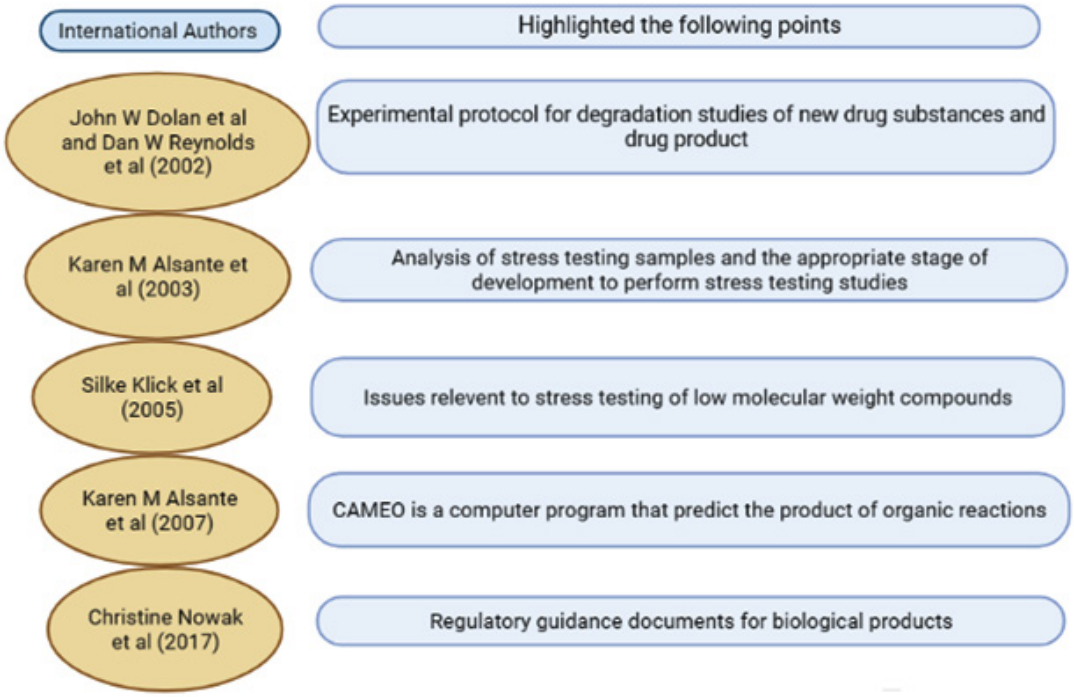

Fig. 1. Highlights of work done by International Authors

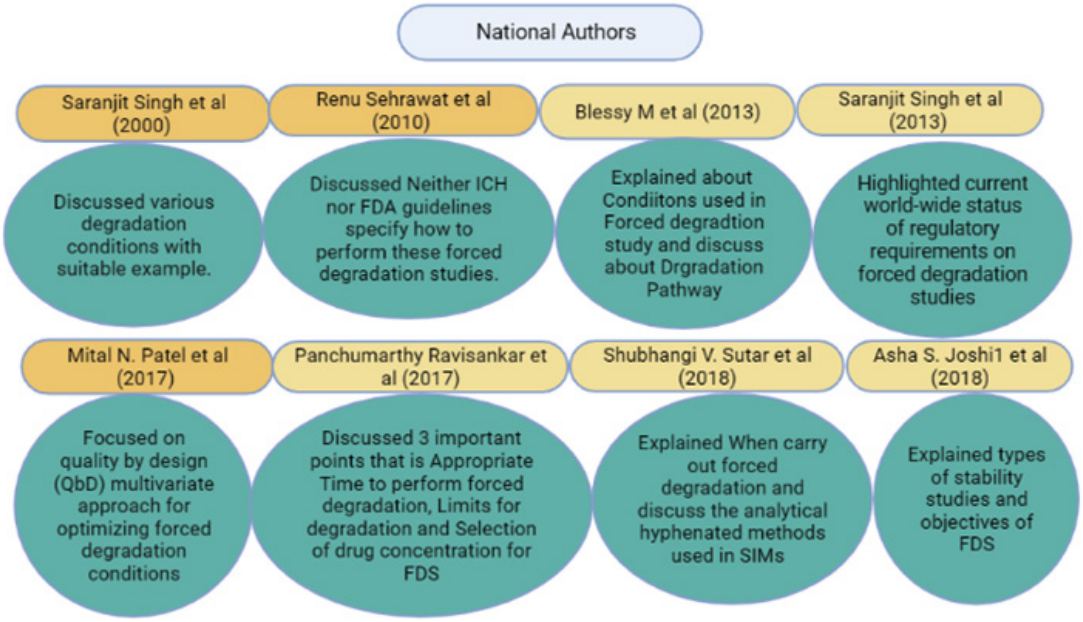

Fig. 2. Highlights of work done by National Authors

sample due to good separation and identification of degrdant sample.[64]

Step involved in development and validation of stability indicating method

Seven steps involve in development and validation of stability indicating method. Following figure 4 shows details steps involve in development and validation of SIMs.

Step I: Study of the drug structure to determine the degradation pathway of drug

This is a first step and very important for establishment of a SIAM. This study provide the information about the structure, by study of the functional groups and other key components. Depending upon functional groups, identification of degradation pathways was carried out. The different functional group shows different chemical reaction like, hydrolysis, Oxidation, thermal and photolytic. Following table 8 shows functional groups and their reactions.[65-67]

Step II: collection of information on physicochemical properties

Before method development steps, it is generally important to know various physicochemical parameters like pKa, log P, solubility, absorptivity and wavelength maximum of

Table 3. Reports of 'stability-indicating' methods where only one stress condition has been employed.[35-40]

Stress condition	Drug	Methodology	References
Oxidation	Nortriptyline and Fluphenazine	UV and HPLC	35
Hydrolysis	Aceclofenac	Spectrophotometric and Densitometric Methods	36
Base hydrolysis	Linezolid	TLC followed by Densitomety	37
Acid hydrolysis	Clozapine	UV, Densitometry, HPLC	38
Hydrolysis	Benoxinate	HPLC analysis with MS detection	39
Hydrolysis	Tinidazole	UV Methods	40

Table 4. Reports of 'stability-indicating' methods where two stress conditions have been employed.[41-48]

Stress condition	Drug	Methodology	References
Hydrolysis both Oxidation	Celecoxib	HPLC	41
Acid hydrolysis	Diloxanidefuroate	UV, Densitometry, HPLC	42
Base hydrolysis	Zafirlukast	LC and derivative spectrophotometry	43
	Sumatriptan succinate	Densitometyspectrophotometric methods	44
	Loratadine	HPLC, densitometricSpectrophotometric	45
	Norfloxacin	capillary electrophoresis	46
Photolytic Thermal	Betamethylepoxide	RP-HPLC	47
Acid hydrolysis Oxidation	Azelastinse and Emedastine	Densitometry TLC	48

Table 5. Reports of 'stability-indicating' methods where three stress conditions have been employed.[49-52]

Stress condition	Drug	Methodology	References
Acidic, alkali and oxidation	Medroxyprogesterone acetate	HPLC	49
	Meloxicam	HPLC	50
Hydrolysis, Photolytic and Thermal	Nitazoxanide	RP-LC	51
Acid, Base, Photolytic	Ebastine	Spectrofluorimetric methods	52

the drug. Following table 9 shows physicochemical properties and Their Importance.

Step III: Force degradation studies

Force degradation studies are carried out in hydrolytic, Oxidative, thermal and photolytic conditions. In hydrolysis, acid and alkali conditions studied by subjecting the drug to various concentration of acid and alkali. If significant degradation is seen then stop analysis of sample. As study time increase it show decrease concentration of active pharmaceutical ingredient. In oxidative conditions, use hydrogen peroxide concentration 3-30%. The drugs should be exposed to light during the photolytic experiments using either a xenon or metal halide lamp, or a mix of cool white and ultraviolet fluorescent lamps. Exposure energy needs to be at least 200 W/m2 of UV and 1.2 million lux/h of fluorescent light, and if decomposition isn't evident, the intensity needs to be increased five times. The medication may be photo stabilized if there is still no breakdown.. In thermal study, Samples of solid- drug substances should be exposed to dry and wet heat. Liquid drug products should be exposed to dry heat. By using Arrhenius equation the Studying a substance's ability to degrade thermally as a function of temperature. The equation are as follows:

$$k = Ae - Ea/RT$$

Where k is specific reaction rate, A is frequency factor, Ea is energy of activation, R is gas constant (1.987cal/deg mol), T is

Table 6. Reports of 'stability-indicating' methods where five (and additional) stress conditions have been employed.[53-57]

Stress condition	Drug	Methodology	References
Thermal, Sunlight Oxidation ,Acid hydrolysis, Base hydrolysis	Felodipine	LC	53
Heat dry, Acid, Base, Neutral, Oxidation, Photolytic	Finasteride	HPLC	54
Acid, Alkali, Oxidation, Dry heat, wet heat treatment, Photolytic	Indinavir	HPTLC	55
Acid, alkali hydrolysis, oxidation Dry heat, wet heat and photolytic	AmiodaroneHCl	HPLC	56
Acid , neutral, BaseOxidation, Photolysis Thermal	Eletriptan hydrobromide	LC and LC-MS	57

Table 7. Reports of 'stability-indicating' methods for Combination of drugs and Pharmaceutical dosage form.[58-63]

Stress Conditions	Drug	Dosage Form	Methodology	References
Hydrolysis Oxidative Photolytic	sumatriptan succinate in pharmaceutical formulations	Tablet	Micellar electrokinetic chromatography	58
Hydrolysis Oxidative Thermal Photolytic	Olmesartan Medoxomile, Amlodipine Besylate and Hydrochlorothiazide	Tablet	RP-HPLC	59
Hydrolysis Oxidative Thermal Photolytic	Metformin Hydrochloride and Sitagliptin Phosphate	Tablet	RP-HPLC	60
Hydrolysis Oxidative Photolytic	Saxagliptin	Tablet	RP-LC-PDA	61
Hydrolysis Oxidative	paracetamol with dantrolene or/and cetirizine and pseudoephedrine	Tablet	RP-HPLC	62
Hydrolysis Oxidative Thermal Photolytic	Hydrochlorothiazide	Oral Suspension	LC	63

absolute temperature. In following figure 5 show experimental conditions, storage conditions and sampling time for FDS.[68]

Step IV: preliminary separation studies on stressed samples

After FDS study stress sample subjected to preliminary analysis. In SIMs mostly chromatographic technique are used. In separation study, selection of column, selection of mobile phase, injection volume, flow rate and run time have important role in proper separation of stress sample from API. For separation of stress sample mostly reversed-phase octadecyl column is used as stationary phase. It shows very good separation of stress sample. Depending upon nature of degradant products the mobile phase will be selected. Initially, water:organic modiûer ratio can be ûxed at 50:50 or can be suitably modiûed so as to obtain proper separation of API and stress sample. For example: In following figure 6 showing separation of febantel and its degradation products.[69]

Step V: Final method development and optimization

After preliminary separation studies,a

Table 8. Functional groups and their reactions

Functional Group	Reaction
Amides, Esters, Lactams, Lactones	Hydrolysis
Thiols, Thioethers	Oxidation
Olefins, Aryl halo derivatives, Aryl acetic acids, and those with aromatic nitro groups, N-oxides	Photocomposition

Table 9. Physicochemical properties and Their Importance

Physicochemical properties	Their Importance
Acid dissociation constants(pKa)	• Selection of mobile phase • Selection of buffer solution
logarithm (base 10) of the partition coefficient (P) (log P)	Provide information on Separation behavior likely to be obtained on a particular stationary phase.
Solubility	• Selection of mobile phase • For analysis of sample
Absorptivity and wavelength maximum of the drug.	• Identification of drug and its degradation products when degradation products are known.

Fig. 3. Techniques used in stability indicating methods

mixture of the reaction solutions is prepared,and subjected again to resolution behavior study. While making this mixture, it is not always necessary to add all reaction solutions withdrawn at different time for all conditions. The method must be optimised by adjusting the mobile phase ratio, pH, gradient, flow rate, temperature, solvent type, the column and its type, in order to separate near or co-eluting peaks.

Step VI: Identiûcation and characterization of degradation products

The identiûcation and characterization of degradation products are initiated once an idea on the nature and number of degradation products formed under different degradation conditions is obtained from preliminary separation studies. In order to identify the resolved product, spectral MS (Mass Spectroscopy), NMR (Nuclear magnetic resonance), IR (Infra-red Spectroscopy) and elemental analysis are used to ascertain its structure. The identity of resolving samples can be determined by HPLC, UV (*Ultraviolet)* detection, full scan mass spectrometry (LC-MS), and tandem mass spectrometry (LC-MS-MS). Molecular weight and fragmentation data are collected using LC-MS or LC-MS-MS, and further in-depth structural data is obtained using LC-NMR analysis.[70-71]

Step VII: Validation of SIMs

ICH guidelines Q2A and Q2B explain Validation of analytical methods. Following other factors like accuracy, precision, linearity,

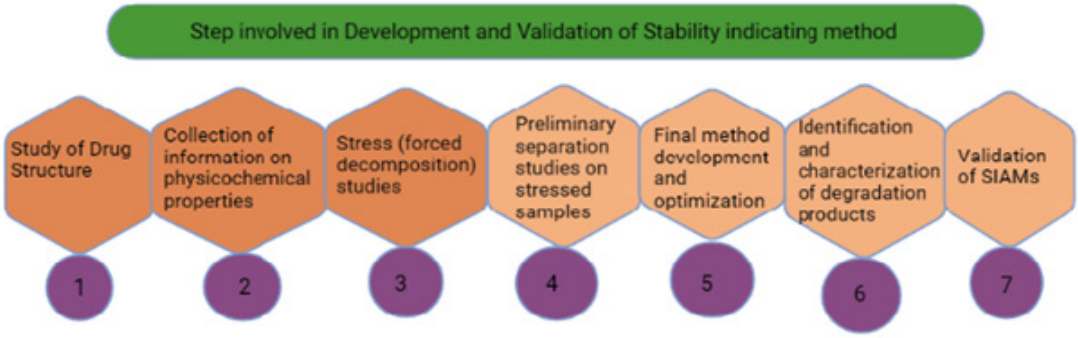

Fig. 4. Step involved in development and validation of stability indicating method

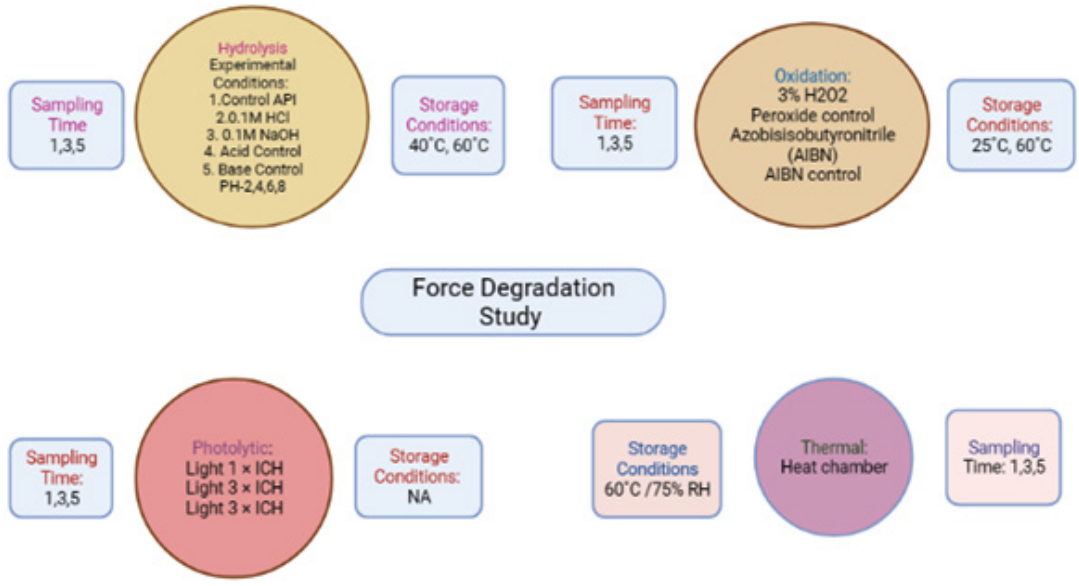

Fig. 5. Force degradation studies

range, robustness, raggedness, etc., the main focus of validation at this point is on establishing the specificity and selectivity of the method. For degradation products, the limits of detection and quantitation are also established in order to aid in the creation of the mass balance. Figure 7 below illustrates a parameter used in the SIMs' validation.[72]

The aspect of mass balance in development of SIAMs

The mass balance is the process of combining the assay value and levels of degradation products to determine if they sum up to exactly 100% of the starting value while taking the analytical error margin into consideration. The ICH parent drug stability guideline defines mass balance as follows. Because it provides a means of determining a SIAM's validity, the mass balance is integrally tied to the creation of a SIAM. If all degradation products are not completely separated, balance cannot be reached. Establishing mass balance becomes easier if only a small number of specific and stable degradation products form, which are simple to separate and for which standards are available. Using standards makes it simple to identify the precise response elements and, consequently, the amounts of the items. Mass balance may, however, be challenging to determine in a variety of circumstances. One or more of the following circumstances may result in this.

• Formation of multiple degradation products, which involve complex degradation pathways and drug excipient interaction.

• Inappropriate detection due to loss of UV chromophore or lack of universal detection.

• If the drug or degradation product are volatile in nature then there will be loss of drugs.

• Diffusive losses into or through containers.

• problems with elution and resolution.

• Due to lack of standards Inappropriate or unknown response factors.

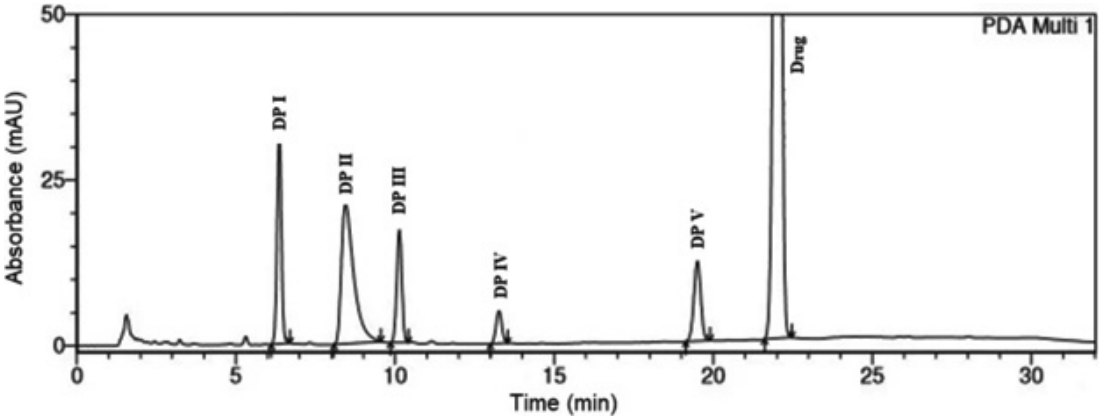

Fig. 6. Chromatogram showing separation of febantel and its degradation products (DP I, II, III, IV, and V in order of elution)

Fig. 7. Validation of SIMs

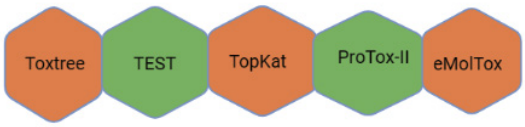

Fig. 8. Software used in toxicity Predication

• In the drug content assay there will be errors and variability.

Problems with establishing mass balance occur if the products are flammable and disappear before the analysis is finished. For instance, metronidazole breaks down into the volatile substances acetic acid and ammonia when it is hydrolyzed in alkali. In fact, the breakdown of medications produces volatile components in a rate of close to 20%. Then there are physical losses such as diffusion into plastic containers, such as nitroglycerin, diazepam, diltiazem, benzyl alcohol, and so on. Even volatile components may be lost through glass bottles due to compound exchange via the closures.[73]

Recent advances in stability indicating methods

Characterization of degradation products: characterization of degradation products is very important to know the structure of degrade sample. But It is difficult task to characterized degrade sample because as time increase their may be the chance of increase number of degradant in sample solution. Recently most advance LC-MS (Liquid chromatography–mass spectrometry) and LC-MS-MS (Liquid Chromatography - Tandem Mass Spectrometry) technique is used in characterization. In many research paper, determination of molecular weight and proper fragmentation pattern was also carried out. Identification of proper fragmentation pattern gives idea about form degradant structure. It is very easy to do further study of degrade sample.

Toxicity study of degradant sample: After characterization of sample, its very important to know their toxicity. Some degrade sample are non-toxic in nature. But some of them are toxic in nature. In recently *in-silico* toxicity study was carried out to study toxicity of degrade sample.[74] Various software are used like Toxtree (toxic hazard estimation), Toxicity Estimation Software Tool (TEST) and TopK at (toxicity prediction by komputer assisted technology), ProTox-II and eMolTox. In toxicity study, mutagenicity, carcinogenicity, skin irritation and eye irritation was determine.

After toxicity study if the degradant molecule not showing any toxicity means the drug molecule is non-toxic in nature. Furthermore do the PASS (Predicted activity spectra for substance) study of that molecule find the activity of molecule it may lead to find new molecular entity.[75]

CONCLUSION

This review is an attempt to explain the several of the examples of drugs given in the tables in the text above, that the stability-indicating assays methods have been developed for a large number of drugs for last several decades, starting almost from 1960s. From 1960 to 2000, the most of them unfortunately fail to meet the current regulatory requirements of stability indicating methods. From year 2000-2019 most of them follow ICH guidelines but still some of them not meet current regulatory guidelines for stability indicating methods. This article focus on regulatory guidelines for SIMs with technique used in force degradation studies and also explain in details development and validation of SIMs.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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