Journal of Chromatography
Separation Techniques

Sharma and Murugesan, J Chromatogr Sep Tech 2017, 8:1
DOI: 10.4172/2157-7064.1000349

Review Article Open Access

Forced Degradation Study an Essential Approach to Develop Stability Indicating Method

Manish Kumar Sharma* and Manoharan Murugesan

Department of Analytical Research and Development, Amneal Pharmaceuticals LLC, Brookhaven, New York, USA

Abstract

Forced degradation studies (stress testing) are very important tool in pharmaceutical research and development to predict long-term stability. Stress studies should be performed in method development to understand drug behavior but also can be performed with method validation for regulatory filling predict stability and measure impurities. For stable formulation development, understanding of chemical behavior, degradation pathways and degradation products of drug substance and drug product is very important. There is not much regulatory guidance available, which can provide step-by-step details on stress testing. Therefore, in present review paper we described the extensive overview of forced degradation studies (stress studies) by specifying strategy to perform stress studies and development of stability indicating method.

Keywords: Forced degradation; Stability indicating method; Mass balance; Peak purity

Introduction

As per FDA guidance, stability indicating test procedure is a validated quantitative analytical procedure that can detect changes in a quality attributes of the drug substance and drug product during storage [1]. Any change in product purity, potency and generation of degradation impurities can be detected with stability-indicating method. A forced degradation or stress study is very crucial step for validation of stability indicating method. It is not always necessary that all impurities generate in real time stability studies will also generate in stress studies but by performing forced degradation studies, degradation impurities can be generate in short period of time. These samples are used to predict long-term stability and develop a stability indicating method. Except photo stability stress testing there is no other guidance available in ICH (The International Conference on Harmonization) Q1B guideline to perform stress testing [2]. Here we propose a practical approach to perform forced degradation study and develop stability indicating method.

The forced degradation studies are performed to,

- Develop and validate a stability indicating method
- Identify degradation products that may be generated during real time stability.
- Develop a method to quantify degradation impurities and separate excipients peaks (placebo) form impurities.
- Interpret possible degradation path-ways
- Generate more stable formulations
- Solve stability-related problems (e.g., mass balance).

Stress conditions

Thermal, hydrolysis, oxidation, and photo degradation are commonly used stress study mechanisms in industry. Desired degradation level can be achieved by selecting suitable concentration of acid, base and oxidizing agent, applying combination stress (e.g., degradation media+temperature) and exposure time. Excess degradation of sample may lead to further degrade, impurities and form secondary degradants that would not be seen in real time stability studies. Excess degradation may mislead mass balance results also because of difference in response factor of unknown impurities. Not achieving desired degradation might not serve the purpose of forced degradation. In recent years complete responses received form FDA, optimum% degradation is should be achieved in all conditions or at least in one condition. In the case of no degradation in all conditions, we should show sufficient scientific effort to achieve degradation. Therefore, it is necessary to control the degradation to a desired level. The generally recommended degradation varies between 5-20% degradation [3,4]. As per industry practice Condition, which commonly used for stress studies are presented in Table 1 [5].

Hydrolysis

Hydrolysis studies are generally carried out in acid and base. In this chemical reaction compound decompose by reacting with water. Acid and base solution are used to perform hydrolysis at wide range of pH (e.g., 2 to 12). For acid hydrolysis hydrochloric acid or sulfuric acids and for base hydrolysis sodium hydroxide or potassium hydroxide are commonly used reagents [6,7]. Concentration of acid or base depends upon stability of molecule. Initially stress study should be performed at room temperature, if desired degradation is not achieved then combination of stress condition (e.g., temperature) can be applied. Acid or base degradation samples should be neutralized with same concentration of base and acid respectively to avoid further degradation reaction and to save HPLC column silica from damage. Alcoholic acid or alcoholic base can also be used for compounds, which are not soluble in water.

Oxidation

Commonly Hydrogen peroxide is used for oxidation degradation of

***Corresponding author:** Manish Kumar Sharma, Department of Analytical Research and Development, Amneal Pharmaceuticals LLC, Brookhaven, New York, USA, Tel: +17327890035; E-mail: mannsharma06@gmail.com

Received January 13, 2017; **Accepted** January 23, 2017; **Published** January 27, 2017

Citation: Sharma MK, Murugesan M (2017) Forced Degradation Study an Essential Approach to Develop Stability Indicating Method. J Chromatogr Sep Tech 8: 349. doi: 10.4172/2157-7064.1000349

Degradation type	Experimental conditions	Storage conditions	Sampling time (Days)
Hydrolysis	Control (no acid or base)	40°C, 60°C	1,3,5
	0.1 M Hydrochloric acid	40°C, 60°C	1,3,5
	0.1 M Sodium Hydroxide	40°C, 60°C	1,3,5
	Acid control (no API)	40°C, 60°C	1,3,5
	Base control (no API)	40°C, 60°C	1,3,5
	pH: 2,4,6,8	40°C, 60°C	1,3,5
Oxidation	3% H_2O_2	25°C, 60°C	1,3,5
	Peroxide control	25°C, 60°C	1,3,5
	Azobisisobutyronitrile (AIBN)	40°C, 60°C	1,3,5
	AIBN control	40°C, 60°C	1,3,5
Photolytic	Light 1 × ICH	Not Applicable	1,3,5
	Light 3 × ICH	Not Applicable	1,3,5
	Light control	Not Applicable	1,3,5
Thermal	Heat chamber	60°C	1,3,5
	Heat chamber	60°C/75% RH	1,3,5
	Heat chamber	80°C	1,3,5
	Heat chamber	80°C/75% RH	1,3,5
	Heat control	Room Temperature	1,3,5

Table 1: Conditions mostly used for forced degradation studies.

drug substance or drug product. Other oxidizing agents such as metal ions, oxygen, and radical initiators (e.g., azobisisobutyronitrile, AIBN) can also be used. Selection of an oxidizing agent, its concentration, and conditions depends on the drug substance [8]. Combination stress (elevated temperature) should not be used with hydrogen peroxide. Chemically hydrogen peroxide is H-O-O-H, in which O-O bonds aren't exactly stable, and hydrogen peroxide does, in fact, decompose at even ambient temperatures. Heating a solution of hydrogen peroxide breaks these bonds even faster. Which leads hydrolysis instead of oxidation. Oxidation degradation samples should be neutralized with same concentration of sodium metabilsulfite solution.

Heat

Thermal degradation test should be performed on active pharmaceutical ingredient and doses form with or without humidity. Solid drug substance, placebo and drug product samples should be exposed to heat with and without humidity, whereas liquid drug substance and drug products can be exposed to heat without humidity. Take extra precaution while applying heat stress to liquid samples if you are going to prepare sample by diluting further (only applicable for injections, oral solutions and syrups), due to heat sample may loss water and concentration of drug substance in solution may change. Information on rate of degradation and primary and secondary degradation products can be understand by generating multiple time points results. In the event of the stress conditions that produce insignificant degradation or not degrade due to stability of molecule, we should apply total energy equivalent to energy applied by accelerated conditions to show sufficient scientific effort to achieve degradation.

Photo stability

This is very important degradation step for light sensitive molecules, but irrespective to light sensitivity, we should evaluated all molecules to identify any unacceptable change due to light exposure. Some recommendations are described in ICH guidlines Q1B Photostability Testing of New Drug Substances and Products [2]. Drug substance, placebo and powdered drug products samples should be exposed to UV-VIS light providing an overall exposure of not less than 1.2 million lux hours and an integrated near ultraviolet energy of not less than 200 watt hours/square meter. It is preferable to expose same samples to cool white fluorescent and near ultraviolet lamp. In the absence of specified instrument, natural light can be used but the spectral distribution as well as the intensity of daylight varies not only with the time of day, weather conditions and atmospheric pollution, which makes natural light not suitable for testing.

Evaluation of Results

Once the forced degradation samples are generated, the next step is their evaluation for the degradation products formed under all the stress conditions. Studying the samples individually does this. Mostly samples are evaluated on chromatographic techniques, the most commonly used are HPLC, UPLC, UHPLC, and CE [9,10]. The major concern here is the successful separation of all the degradation products from the drug and from each other, viz development of stability-indicating method [11]. Hence, preference has to be given to a selective method, with emphasis on peak purity. Sensitivity of the developed analytical method should have well enough to detect impurities at low levels. During the method development it may happen that the drug peak may hide a degradation impurity peak that co-elutes with the drug. All known and unknown impurities should be well separated from main component peak and all unknown impurities should be well separated from known impurities. Sometime two merged unknown impurity may lead to out of specification results in real time stability therefore analytical method should have capability to resolve any unknown impurities higher then unknown impurity specification level from merging with each other.

Peak purity analysis

Peak purity parameters are used to prove spectral uniqueness of compound. Peak purity of main component and known impurities in control and degradation samples should be established using PDA (Photo diode array) detector. There are some limitations in establishing of peak purity and cannot be calculated accurately if,

1. UV detection response of main component at saturation level (Non linear UV detection at higher absorbance values)

2. Co-eluting peak having same UV spectra.

3. Co-eluting peak has no chromophoric functional group.

For further investigation other technique can be use to establish peak purity like LC-MS.

Mass balance

In recent years FDA strictly asking mass balance in forced degradation of related compound method to prove specificity and capability to quantify degradation impurities. Mass balance establishes adequacy of a stability indicating method though it is not achievable in all circumstances. Lacking of mass balance lead a suspicion on capability of method to quantify all degradation products accurately. Mass balance is always challenging to evaluate accurately. The mass imbalance, apart from varying responses of analyte and degradation product peaks, may also happen due to potential loss of volatile degradation products, formation of non-chromophoric compounds, formation of early eluents, and retention of compounds in the column [12] The known impurity standards can be used to calculate a mass balance more accurately, as their availability helps in the quantitative determination through corrected response factors. A popular formula used in industry for mass balance is [12]:

[100-(water by KF+total volatiles+residue on ignition+% of non-chromophoric contents)] × HPLC purity/100

Conclusion

Forced degradation studies (stress testing) are very important tool in pharmaceutical research and development to develop stable formulation. It provides information about degradation pathways of drug substances and drug products, which can be used in excipient compatibility and provide help in early stage development. Degradation impurities generated in stress studies may or may not generate in real time stability study but this important step is used primarily to develop stability- indicating analytical methods. It is strongly recommended that these studies should be started as early as possible to be able to provide valuable information that can be used to assess the inherent stability of a drug and to improve formulation and the manufacturing process.

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