SOP FOR FORCED DEGRADATION STUDY

1.0 OBJECTIVE:

This document describes the procedure for performing forced degradation study of drug products (Formulations)/ drug Substances (APIs).

2.0 SCOPE:

This SOP is applicable to Assay and Related Substances test performed for drug products/ drug substances by HPLC using UV detector and by TLC method, performed in Quality Control & Analytical development (ADL) Laboratory.

3.0 RESPONSIBILITY:

Analyst is responsible for

- Preparation of Forced Degradation Study Protocol, Method details, Experimental design, Test data sheet and report as specified in this document.
- Execution of the procedure specified in protocol for the study.
- Compilation and filling of the study documents.
- Documentation of any non-conformance observed during study.

Section Head / Reviewer is responsible for

- Review of Forced Degradation Study Protocol, Experimental Design, Data Sheet and Report.
- Investigation and action on any non-conformance observed during Forced Degradation Study.
- Monitoring that the procedure for forced degradation study of drug substances and drug products is followed as per procedure specified in this SOP.

Head Quality Control is responsible for :

- Verification of Forced Degradation Study Protocol and Report.
- Ensuring that the procedure for forced degradation study of drug substances and drug products is followed as per procedure specified in this SOP.

The QA Dept. is responsible for:

- Issuance of study data sheets.
- Verification of Forced Degradation Study Protocol and Report.
- Ensuring that the procedure for forced degradation study of drug substances and drug products is followed as per procedure specified in this SOP.

- Approval of Forced Degradation Study Protocol and Report.

4.0 ACCOUNTABILITY:

Head QC/Head ADL

5.0 PROCEDURE:

6.0 DEFINITIONS:

6.1 Forced degradation study: Forced degradation study is carried out to identify the likely degradation products, which can establish degradation pathways, the intrinsic stability of the molecule and evaluate the specificity and stability indicating power of the analytical procedures used.

6.2 Placebo: A mixture of ingredients of formulation without the analyte under determination.

6.3 Forced degradation study is to be performed for drug products (wherever applicable) manufactured /drug substances (as per requirement).

6.4 Forced degradation study is to be performed to achieve purposeful degradation that is predictive of long-term and accelerated storage conditions. Hence, selecting suitable reagents such as concentration of acid, base, or oxidizing agent and varying the conditions and length of exposure which can achieve the preferred level of degradation is essential.

6.5 In forced degradation study, application of over stress conditions may lead to formation of secondary degradants that may not be seen in formal shelf-life stability studies and under stressing may not serve the purpose of stress testing. Hence, it is necessary to control the degradation to a desired level. The degradation should be generally achieved between 5% to 15 %. **Even after several extended attempts, if the degradation observed is not within 5% to 15%, report the result as final**.

6.6 If literature data of a molecule is available to support that the molecule does not undergo degradation in solution form i.e. acid hydrolysis / base hydrolysis / oxidation / hydrolysis, in such a case, study can be performed by treating sample at extreme stress condition e.g. Addition of 5M HCl and heating for 30 minutes.

6.7 Photolysis/Photo stability: The samples to be exposed to both white and UV light with the requirement of minimum of 1.2 million lux hours and 200 watt hours per square meter. It is necessary to maintain the temperature during this exposure.

6.8 The recommended pH range (working pH range) of the column also to be considered during the study.

6.9 The **Oxidative degradation** being complex, the selection of an oxidizing agent, its concentration, and conditions depends on the drug substance / product. Although hydrogen peroxide is used predominantly because it mimics possible presence of peroxides in excipients.

6.10 Hydrolysis is one of the most common degradation chemical reactions over wide range of pH. In Hydrolysis, drug reacts with water to yield break down products of different chemical compositions. Water either as a solvent or as moisture in the air comes in contact with pharmaceutical dosage forms and is responsible for degradation of most of the drugs. Hydrolysis of most of the drugs is dependent upon the relative concentration of hydronium and hydroxyl ions.

6.11 Peak Purity analysis : Peak purity is used as an aid in stability indicating method development. The spectral uniqueness of a compound is used to establish the peak purity when co-eluting compounds are present. It is necessary to verify the peak height for establishing spectral purity. As UV detection may not be linear at higher absorbance values, threshold should be set such that co-eluting peak can be detected.

6.12 Mass balance : Mass balance establishes adequacy of a stability indicating method though it is not achievable in all circumstances. It is calculated by adding the assay value and the amounts of impurities and degradants to evaluate the closeness to 100 %. Attempt should be made to establish a mass balance for all stressed samples. Possibly any imbalance should be explored.

Formula for Mass Balance calculation as below :

$$\text{Mass balance} = \frac{(\%\ \text{Assay} + \%\ \text{Total impurities of Stressed sample}) \times 100}{(\%\ \text{Assay} + \%\ \text{Total impurities of Protected sample})}$$

Note: In case of drug products, the placebo should also be subjected to the same degradation conditions along with the sample.

6.13 For the forced degradation study, prepare protocol for the same stating all the details regarding the study to be conducted. Allocate a protocol number for the degradation study as follows :

e.g. FDS/XX/YY/T

Where,

FDS : Forced Degradation study.

XX : Unit identity. e.g. DS for Diclofenac Sodium

YY : 'DS' in case of Drug Substance (Active Pharmaceutical Ingredient) and 'DP' in case Drug Product (Finished Product).

T : 'T' is the alphabet for identification of test (A : Assay, I : Impurity). In case of more degradations for a test e.g. Assay, use as A1, A2, A3 etc.

6.14 If any regulatory / customer demands monitoring of any other stress condition, perform the same accordingly as per requirement.

6.15 The powder samples (drug substances) and solid dosage forms (e.g. Tablets, capsules etc.), should be subjected to photolysis 'as such' in powder form and should not be treated in solution form. For liquid / semisolid drug substances and drug products, the sample is to be subjected 'as such' in a suitable container (e.g. glass beaker / petridish) for photolysis study.

Note: Treat the blank / placebo in same manner as that of sample.

6.16 For direct scale up products of multiple strengths, which are manufactured through the same standardized process with weight proportionate and same matrix, perform degradation study on one strength (preferably lower strength). In case of multiple strength products which are not manufactured through the same standardized process with weight proportionate and same matrix, select the drug products of minimum and maximum strength among all the strengths for forced degradation study.

6.17 While performing the forced degradation study, the container (e.g. Petridish, Iodine flask, Reflux assembly etc.) container used for photolysis should be transparent.

6.18 If the drug substance has no solubility in the aqueous acidic / basic medium, then an organic solvent such as methanol / ethanol / acetonitrile etc. can be used to dissolve the material. For oxidation purpose a solution of hydrogen peroxide in acetonitrile can be used.

6.19 In case of normal phase analysis, prepare the forced degradation reagents like HCl, NaOH etc. in suitable organic solvents like methanol / ethanol / acetonitrile etc., to avoid the probable deterioration of HPLC column due to water, if used.

6.20 The analysis of the treated sample should be performed concomitantly with that of protected sample (untreated sample).

6.21 Review the difference in area of principal peak of protected sample to exposed sample to understand % degradation occurred. The % degradation of principal peak can be calculated by following formula :

% degradation = Peak area response of principal peak of Stressed sample x 100
Peak area response of principal peak of Protected sample

6.22 For the test of Assay, the chromatographic run time must be kept covering the last eluting impurity or 1.5 the retention time of main component, whichever is greater. For impurity determination, unless otherwise specified, run the sample for at least three times the retention time of the last eluted principal peak of the drug substance.

6.23 Refer below table for the "Conditions" for the forced degradation study:

"Conditions" for Forced Degradation Study :

Drug Substance / Drug Product	As such sample Degradation		Degradation of Sample In solution form			
	Elevated temperature	Photolysis	Acid hydrolysis	Base hydrolysis	Oxidation	Hydrolysis
Solid	Yes	Yes	Yes	Yes	Yes	Yes
Liquid	Yes	Yes	Yes	Yes	Yes	NA
Semisolid	Yes	Yes	Yes	Yes	Yes	Yes

NA : Not applicable

6.24 Degradation Criteria :

6.24.1 If degradation is achieved more than 15 % complete degradation is achieved.

6.24.2 If degradation achieved is less than 5% or if no degradation is achieved : Additional attempts to be made to achieve the degradation between 5% to 15%. The same can be done by gradually increasing the time / concentration / conditions / refluxing / treatment etc. as per the parameter under study. Additional attempts by suitably varying the time / concentration / conditions / refluxing / treatment etc. to be carried out before concluding the non-susceptible nature of the molecule to the condition under study.

Note: Even after several extended attempts, if the degradation observed is not within 5% to 15%, report the result as final.

6.24.3 During the course of the study if the degradation between 5% to 15% is achieved, no further attempts for the respective parameter under study are required and the study to be stopped / terminated and the findings to be reported.

6.24.4 Protected sample : The sample used as reference sample along with its Primary and Secondary Packaging Material or the sample which is not exposed to any condition and stored as per the recommended storage condition mentioned in the product labeling.

6.24.5 As such sample" degradation :As such sample' degradation consists of following stress conditions:

a) Elevated temperature

b) Photolysis

6.24.6 Elevated Temperature: This consist of below mention condition :

Dry Heat: Sample to be treated to a temperature of 80°C. If melting point of the drug substance is about 80°C or less then carry out degradation at a temperature which is 20°C lower than the melting point of the drug product.

- The 24 hours treatment of sample should be preserve ,so that it will be available for analysis along with 48 hours treatment sample.
- After completing 24/48 hours treatment, remove the exposed samples, protect from moisture, light and store at room temperature.
- Analyze the 48th hour sample and determine whether total degradation is between 5% to 15%.
- If the degradation is more than 15 %, then analyze the 24th hour sample. For increasing or decreasing the degradation, the time period may be reduced or increased to achieve desired degradation.

Wet Heat: Preferably, sample to be subjected or moist heat sterilization at a temperature of 121°C for a time period of 30 minutes or as per validated process time (reduced or increased).Analyze the 30 minutes sample and determine whether total degradation is between 5% to 15%.

- For increasing or decreasing the degradation, the time period may be reduced or increased to achieve desired degradation.

Photolysis: For this wherever required the samples should be treated to light providing

an overall illumination of 1.2 million LUX hours and an integrated near ultraviolet energy of not less than 200 watt hours / sq. meter (option II as per ICH guideline)

- Analyze the treated sample and determine whether total degradation is between 5% to 15%.

6.25 Degradation of sample in Solution form: Solution form degradation consists of following stress conditions:

a) Acid hydrolysis
b) Base hydrolysis
c) Oxidation
d) Hydrolysis

Note : Hydrolysis stress condition is not applicable for normal phase chromatographic analysis.

a) <u>Acid hydrolysis</u>:

- Transfer appropriate amount of sample in iodine flask or round bottom flask and add 5 ml to 10ml 1 M HCI (increase the volume if 5 ml to 10 ml is insufficient)

  Treat the sample on a boiling water bath for two hours. At the end of exposure, cool the solution and transfer in an appropriate volumetric flask and dilute with diluent up to the mark to get the required concentration as per the 'Method Details' or as per experimental design, analyze the solution. Determine whether total degradation is between 5% to 15%.

- If the degradation is more than 15%, then prepare a new sample solution and treat it for one hour and test this one hour sample. If degradation is more than 15 % even for one hour sample then treat the sample with diluted solution of HCl (e.g. 0.1M or 0.01M HCl) calculated in such a way to obtain the degradation between 5% to 15%, or check the degradation without refluxing or by further reducing the refluxing time. For the sample treated with diluted solution or without reflux, if the degradation is between 5% to 15% then report the result as final.

- If degradation is less than 5% or no degradation is observed, for the treated sample then subject the sample to higher concentration of the acid or alternatively increase the refluxing time from 2 hours to 3 hours (or even higher), so as to obtain the degradation between 5% to 15%. If the degradation is between 5% to 15% then report the result as final.

a) Base hydrolysis:

- Transfer appropriate amount of sample in iodine flask or round bottom flask and add 5 ml to 10 ml of 1 M NaOH (increase the volume if 5 ml to 10 ml is insufficient).

Reflux the sample on a boiling water bath for two hours. At the end of exposure, cool the solution and transfer in an appropriate volumetric flask and dilute with diluent upto the mark to get the required concentration as per the 'Method Details' or as per experimental design, analyze the solution. Determine whether total degradation is within 5% to 15%.

- If the degradation is more than 15%, then, prepare a new sample solution and reflux it for one hour and test this one hour sample. If degradation is more than 15% even for one hour sample then treat the sample with diluted solution of NaOH (e.g. 0.1M or 0.01M NaOH) calculated in such a way to obtain the degradation between 5% to 15%, or alternatively check the degradation without refluxing or by further reducing the refluxing time. For the sample treated with diluted solution or without reflux, if the degradation is between 5% to 15% then report the result as final.
- If degradation is less than 5% or no degradation is observed, for the treated sample then subject the sample to higher concentration of the base or alternatively increase the refluxing time from 2 hours to 3 hours (or even higher), so as to obtain the degradation between 5% to 15%. If the degradation is between 5% to 15% then report the result as final.

C) Oxidation:

- Transfer appropriate amount of sample in iodine flask or round bottom flask and add 5 ml to 10 ml of 10 % hydrogen peroxide (increase the volume if 5 ml to 10 ml is insufficient). Reflux the sample on a boiling water bath for two hours. At the end of exposure, cool the solution and transfer same in an appropriate volumetric flask and dilute with diluent up to the mark to get the required concentration as per the specification or as per experimental design, analyze the solution. Determine whether total degradation is between 5% to 15%.
- If the degradation is more than 15%, then prepare a new sample solution and reflux it for one hour and test this one hour sample. If degradation is more than 15% even for

one hour sample then treat the sample with diluted solution of Hydrogen peroxide (e.g. 5% or 3% solution of Hydrogen peroxide) calculated in such a way to obtain the degradation between 5% to 15%, or check the degradation without refluxing or by further reducing the refluxing time. For the sample treated with diluted solution or without/reduced refluxing time, if the degradation is between 5% to 15% then report the result as final.

Note : Based on sensitivity of molecule, desired degradation can be achieved by means of mild oxidation which involves purging of solution by using air / oxygen.

- If degradation is less than 5% or no degradation is observed for the treated sample then subject the sample to higher concentration of the oxidizing agent or alternatively increase the refluxing time from 2 hours to 3 hours (or even higher), so as to obtain the degradation between 5% to 15%. If the degradation is between 5% to 15% then report the result as final.

D) <u>Hydrolysis</u>:

- Transfer appropriate amount of sample in iodine flask or round bottom flask and add 5 ml to 10 ml of purified water (increase the volume if 5 ml to 10 ml is insufficient) .Reflux the sample on a boiling water bath for two hours. At the end of exposure, cool the solution transfer in an appropriate volumetric flask and dilute with diluent up to the mark to get the required concentration as per the specification or as per experimental design, analyze the solution. Determine whether total degradation is within 5% to 15%.
- If the degradation is more than 15%, then, prepare a new sample solution and reflux it for one hour and test this one hour sample. If degradation is more than 15% even for one hour sample then treat the sample for the reduced time period calculated in such a way to obtain the degradation between 5% to 15%, or check the degradation without refluxing or by further reducing the refluxing time. For the reduced time sample or without reflux, if the degradation is to obtain the degradation between 5% to 15% then report the result as final.
- If degradation is less than 5 % or no degradation is observed, for the treated sample then subject the sample by increasing the refluxing time from 2 hours to 3 hours (or even higher), so as to obtain the degradation between 5% to 15%. If the degradation is between 5% to 15% then report the result as final.

6.26 Interpretation and Reporting of Results :

Report the actual description of the treated samples and compare with that of a protected sample and report the results as 'No significant change' if the description is comparable, and as 'Significant change' if the description is not comparable. Based on the interpretation of data, derive and summarize the conclusion in the Forced Degradation Study Report.

6.27 Documentation:

SOP related soft copies and hard copies shall be retained by respective department.

7.0 ABBREVIATIONS:

HPLC	High Performance Liquid Chromatography
ICH	International Conference on Harmonization
LOD	Limit of Detection
LOQ	Limit of Quantification
NLT	Not less than
NMT	Not More than
No.	Number
PDA	Photodiode Array **(**PDA) Detector
QA	Quality Assurance
QC	Quality Control
RSD	Relative standard deviation
USP	United State of Pharmacopoeia

8.0 ANNEXURES:

ANNEXURE No.	TITLE OF ANNEXURE	FORMAT No.
Annexure -I	Forced Degradation Study Protocol	

9.0 DISTRIBUTION:

- Master Copy — Quality Assurance Department
- Controlled Copy No. 01 — Head Quality Assurance
- Controlled Copy No. 02 — Head Quality Control

10.0 REFERENCES:

ICH Q1B: Stability Testing: Photo stability Testing of New Drug

11.0 REVISION HISTORY:

Revision No.	Change Control No.	Details of Changes	Reason of Changes	Effective Date	Done By
00	Not Applicable	Not Applicable	New SOP		