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Basic Concept of Stability Profile and Stress Degradation Pathway of Pharmaceutical Formulations: A Review

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ABSTRACT: Constrained corruption is a debasement of new medication substance and medication item at conditions more serious than quickened conditions. It is required to show particularity of soundness demonstrating strategies and furthermore gives an understanding into debasement pathways and corruption results of the medication substance and aides in illustration of the structure of the debasement items. Constrained corruption ponders demonstrate the substance conduct of the atom which thusly helps in the advancement of definition and bundle. Moreover, the administrative direction is extremely broad and does not clarify about the execution of constrained corruption ponders. A fundamental advance in the structure of an administrative agreeable solidness program for medication and formalized as an administrative necessity. Subsequently, this survey examines the present patterns in execution of constrained corruption ponders by giving a methodology to directing examinations on corruption components and furthermore portrays the explanatory techniques. Basically stability indicating assay or forced degradation studies accompanied by photolytic, hydrolytic, acidic, alkali and oxidative routes even complete studies performed under the prescribed guideline of ICH. In this review we are trying to focus on various routes of degradation study and ICH guideline to establish stability indicating assay.

Keywords: Stress Degradation; Stability Indicating Assay; Photolytic Degradation; Hydrolytic Degradation; Oxidative Degradation; Acidic Degradation; Alkali Degradation; ICH Guideline.

INTRODUCTION: Unwanted chemicals present in pharmaceuticals with the APIs (Active Pharmaceutical Ingredient's) or those is develop during formulation or upon ageing of both API and formulation is called as impurities.[1] The presence of unwanted chemicals even in very small amount may influence the safety and efficacy of pharmaceutical product. The process in which the natural degradation rate of a pharmaceutical product is increased by the application of an additional stress is called as forced degradation study. Forced degradation studies generally involve the exposure of representative samples to the relevant stress conditions like heat, humidity, acid or base hydrolysis, and oxidation.[2] The main reason of performing stability studies is to provide proof in the form of stability data that how quality of a pharmaceutical product or drug varies with environmental factors, i.e., temperature, light, moisture, etc. This stability data can also be used to determine a re-test period of drug substance and also to calculate the shelf life of a product and its favorable storage conditions. The stability of a drug substance or a drug product is a critical parameter which may affect purity, potency, efficacy and safety. If the stability of drug changes which form toxic degradation products or deliver a lower dose than expected and due to this which is dangerous to patient safety. Therefore it is essential to know the purity profile and behavior of a drug substance under various environmental conditions.

Rationale for Reporting of Impurity: Now a day not only purity profile but also impurity profile has become essential as per various regulatory requirements. Any chemical present in a drug substance other than itself or in a drug product and ingredient called as impurity. It may be formed during synthesis of drug substance or formed due to degradation of drug substance or reaction between drug substance and excipient. Hence impurity may be two types either process related impurity (PRI) which includes starting materials, reaction intermediates, chemical reagents, ligands and catalysts, by-product of synthetic route and residual solvents or degradation related impurity (DRI) which is formed due to hydrolysis, oxidation or photolysis of drug. The main purpose of impurity identification and quantification is to provide information on how the quality of a drug substance or drug

product varies under the influence of degradation related factors such as temperature, humidity and light and process related factors such as pH, solvents, or reagents which are used in synthetic process.[3]

Classification of impurities: Impurities have been named differently or classified as per the ICH as follows; [4,5]

a) Common names:

i. By-products
ii. Degradation products
iii. Interaction products
iv. Intermediates
v. Penultimate intermediates
vi. Related products
vii. Transformation products

b) United State Pharmacopeia: Classification of impurities according to United States Pharmacopoeia (USP);

i. Impurities in Official Articles
ii. Ordinary Impurities
iii. Organic Volatile Impurities

c) ICH Terminology: Classification of impurities according to United States Pharmacopoeia (USP);

i. Organic Impurities (Process and Drug related)
ii. Inorganic Impurities
iii. Residual Solvents

Reason behind forced degradation study [6]: Forced degradation studies are carried out for the following reasons:

- To determine degradation pathways of drug substances and drug products (e.g., during development phase)
- To understand the drug molecule chemistry
- To generate a degradation profile that mimics what would be observed in a formal stability study under ICH conditions
- To solve stability-related problems (e.g., mass balance)
- To observe effect of environmental conditions on drug/drug product.
- To predict shelf life of a drug product.
- To establish storage conditions of a drug product.
- To determine labeling instructions of drug product (expiration dating).
- To establish re-test period of a active pharmaceutical ingredient (API).
- To develop a stability profile of a drug substance.

TYPES OF STABILITY STUDIES: As per ICH guidelines different types of stability studies are

1. Real time stability studies or Long term stability studies (room temperature and humidity conditions)
2. Intermediate stability studies
3. Accelerated stability studies (high heat and humidity conditions

Real Time Stability Studies: Real time stability studies are performed from at least 12 months to allow significant degradation pathway.

Intermediate stability studies: Intermediate stability studies are performed from at least 6 months to allow significant degradation pathway.

Accelerated Stability Studies: In Accelerated stability studies the rate of chemical degradation or physical changes is increase by using exaggerated conditions, like high temperature and humidity.[7]

These studies are performed for the following purposes:

1. To assess long term chemical effects observed at non-accelerated conditions.
2. To evaluate the effect of short deviations from labeling storage conditions as might occur during shipping.

Table 1: Sampling Time Points in Various Stability Studies.

Type of Study	Sampling Time Points (in months)
Long term	0, 3, 6, 9, 12, 18, 24
Intermediate	0, 3, 6, 9, 12
Accelerated	0, 3, 6

For accelerated stability studies a minimum of 3 time points are required. For example, for a 6 month study the three time points are 0, 3 and 6 months, respectively. In case of exceptions where results from 3 time points are likely to change significantly or vary to a significant level, intermediate studies come into existence and additional testing may be performed by adding more samples or by including fourth time point.[7] The ICH guideline [Q1A (R2)] clearly defines the significant change. 'Significant change' refers to:

1. A 5% shift in assay value from its initial value or inability to remain within defined limits in case of biological and immunological procedures.
2. Any degradation product exceeding its specified limit values.
3. Changes in appearance, physical attributes and functionality test including color, phase separation,

resuspendibility, caking, hardness, dose per actuation but excluding attributes such as softening of suppositories, melting of creams.

4. Change in pH value more than the specified limits.

5. Not meeting dissolution limits for 12 dosage units.

The concept of accelerated stability testing is based upon the Arrehenius equation: [8]

$InK = InA + \Delta E/RT$

Where,

K = Rate constant of a chemical reaction,

A = Pre-exponential factor, ΔE = Activation energy,

R = Gas constant or Boltzmann constant,

T = Absolute temperature (in kelvin).

Overview of regulatory guidance: The stability studies are conducted according to guidelines issued by WHO, ICH, FDA or EMEA.[9] Table 2 lists major guidelines for stability studies.

Table 2: Major Guidelines for Stability Studies.

Sr. No.	Stability Study Guideline	Reference
1.	ICH guidelines	http://www.ich.org/products/guidelines/quality/article/qualiy-guidelines.html
2.	FDA guidelines	http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/ Guidance for Industry
3.	WHO guidelines	http://www.ich.org/fileadminPublic_Web_Site/ICH_Products/Guidelines/ Quality/Q1F/Stability_Guideline_WHO.pdf

ICH guidelines: It includes,

- Stability - Q1A – Q1F
- Analytical Validation – Q2
- Impurities – Q3A - Q3C (Q3D – concept paper)
- Pharmacopoeias – Q4A - Q4B (and annexes)
- Quality of Biotechnological Products – Q5A – Q5E
- Specifications – Q6A – Q6B
- Good Manufacturing Practice – Q7
- Pharmaceutical Development – Q8
- Quality Risk Management - Q9
- Pharmaceutical Quality System – Q10
- Development and Manufacturing of Drug Substances – Q11

Table 3: ICH Guidelines for Stability Studies.

Sr. No.	ICH Guideline Code	Title
1.	Q1A	Stability testing of new drug substances and products
2.	Q1B	Stability testing : Photostability testing of new drug substances and products
3.	Q1C	Stability testing of new dosage forms
4.	Q1D	Bracketing and matrixing design for stability testing of drug substances and products
5.	Q1E	Evaluation of stability data
6.	Q1F	Stability data package for registration applications in climatic zone III and IV
7.	Q5C	Stability testing of biotechnological/biological products

Strategy for selection of degradation conditions: Forced degradation is carried out to produce representative samples for developing stability-indicating methods for drug substances and drug products. Selection of stress conditions should be depends on the product's decomposition under normal manufacturing, processing, storage, and use conditions for stress which are specific in each case.[10] A general protocol of degradation conditions used for drug substance and drug product is shown in fig.1.

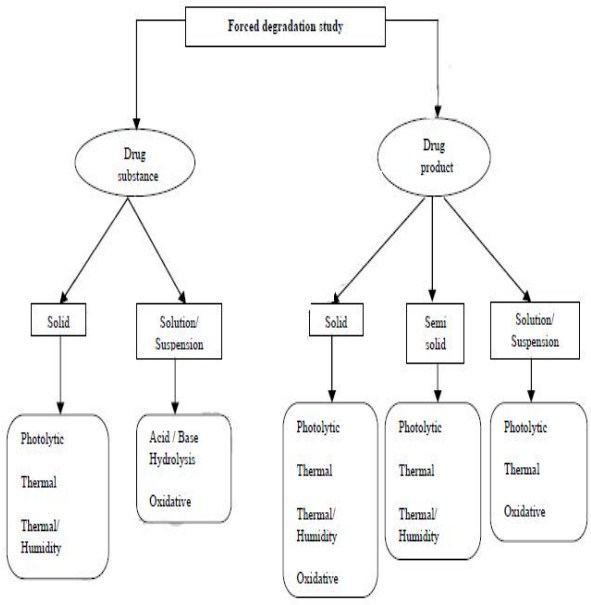

Figure 1: overview of forced degradation.

A list of stress factor included for forced degradation studies are acid and base hydrolysis, thermal degradation, photolysis, oxidation.[11-14] and may include freeze-thaw cycles and shear.[15]There is no specific procedure in regulatory guidelines about the Conditions of pH, temperature and specific oxidizing agents

used in forced degradation study. The outline of photolysis studies is left to the applicant's discretion although Q1B specifies that the light source should produce combined visible and ultraviolet (UV, 320-400 nm) outputs, and that exposure levels should be justified. [16] The initial trial should have the aim to come upon the conditions that degrade the drug by approximately 5-20%. Some conditions which mostly used for forced degradation studies are reported in Table 4.[17]

Degradation conditions:

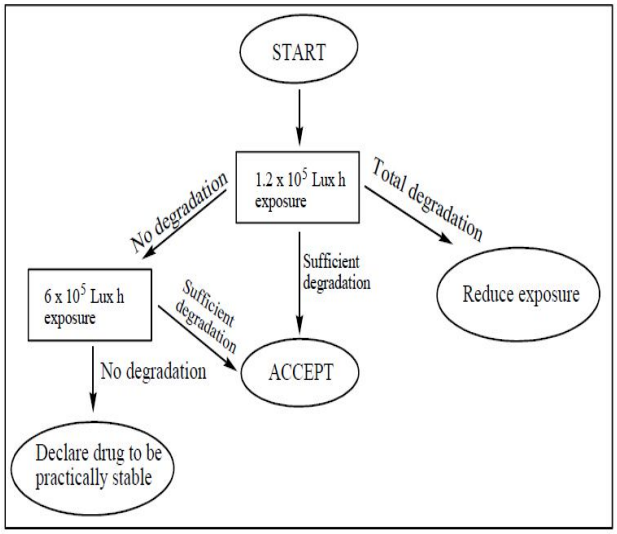

Figure 2: Flow chart of photolytic degradation.[18]

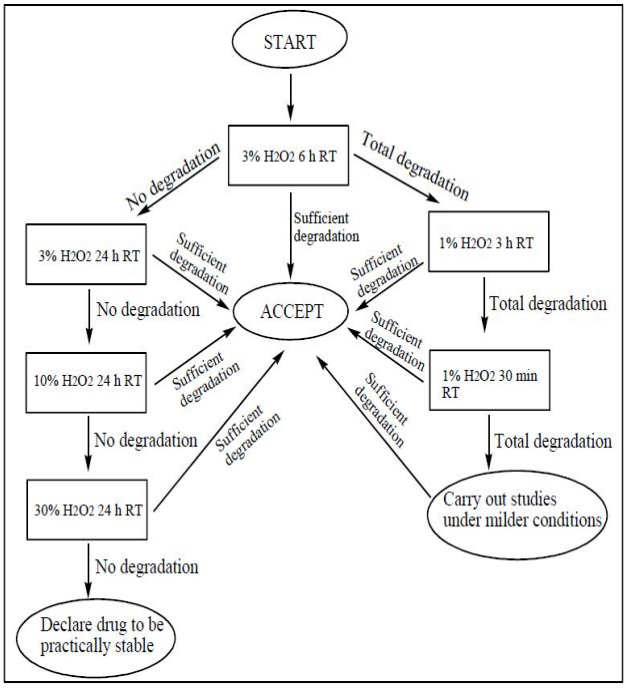

Figure 3: Flow chart of oxidative forced degradation.[18]

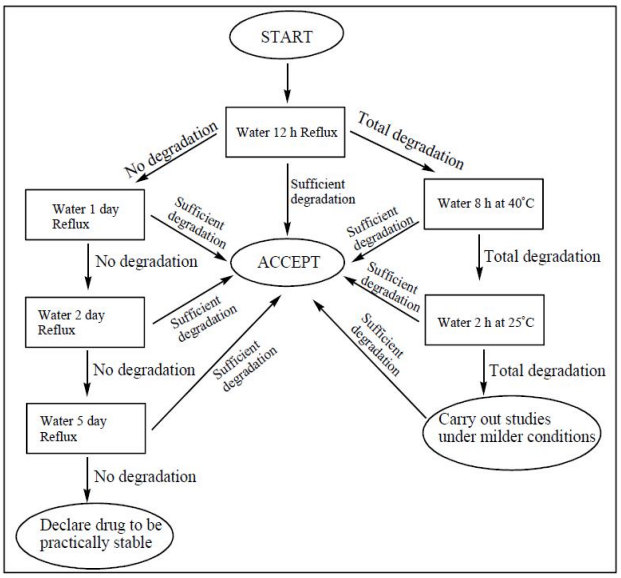

Figure 4: Flow chart of Neutral forced degradation[18].

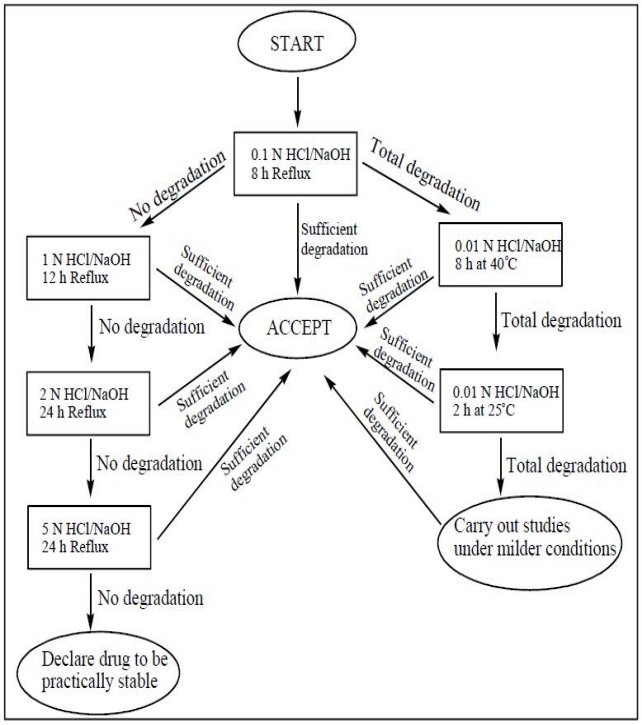

Figure 5: Flow chart of acid/alkali induced hydrolysis.[18]

Stability indicating method: A stability indicating method (SIM) is an analytical procedure which is used to quantitate the decrease amount of the active pharmaceutical ingredient (API) present in drug product due to degradation. According to FDA guidelines, a stability-indicating method is a validated quantitative analytical procedure that can be used to detect how the stability of the drug substances and drug products varies with time. A stability-indicating method accu-

rately quantitates the changes in active ingredients concentration without interference from other degradation products, impurities and excipients [19]. Stress testing is carried out to demonstrate specificity of the developed method to measure the changes in concentration of drug substance when little information is available about potential degradation product. The development of a suitable stability indicating method provides a background for the pre-formulation studies, stability studies and the development of proper storage requirements. Singh and Bakshi [20] discussed some critical issues about developing stability indicating methods. Dolan [21] made comments and suggestions on stability indicating assays. Smela [22] discussed from regulatory point of view about stability indicating analytical methods. The RP-HPLC is most widely used analytical tool for separation and quantitation of the impurities and it is most frequently coupled with UV detector [23]. The steps involved for development of SIM on HPLC which meets the regulatory requirements are as follows:

a. Sample generation: For generating samples for Stability Indicating Method the drug is force degraded at conditions more severe than accelerated degradation conditions. It involves degradation of drug at hydrolytic, oxidative, photolytic and thermal conditions as discussed in degradation condition. The forced degradation of API in solid state and solution form is carried out with an aim to generate degradation products which are likely to be formed in realistic storage conditions This sample is then used to develop anSIM.[24]

b. Method development and optimization: Before starting method development, various physiochemical properties must be known like pKa value, log P, solubility and absorption maximum of the drug for HPLC method development. Log P and solubility helps in selection of mobile phase and sample solvent while pKa value helps to determine the pH of the mobile phase [18]. Reverse phase column is a first choice to start the separation of sample components as the degradation is carried out in aqueous solution. Various solvent methanol, water and acetonitrile can be used as mobile phase in various ratios for the initial stages of separation. Selection between methanol and acetonitrile for organic phase is based on the solubility of the analyte. Initially the water: organic phase ratio can be kept at 50: 50 and suitable modifications can be made as trials proceed to obtain a good separation of peaks. Latter buffer can be added if it is required to obtain better peak separation and peak symmetry. If the method is to be extended to LC-MS then mobile phase buffer should be MS compatible like trifluoroacetic acid and ammonium formate. Variation in column temperature affects the selectivity of the method as analytes respond differently to temperature changes. A temperature in the range of 30-40°C is suitable to obtain good reproducibility. It is better to push the drug peak farther in chromatogram as it results in separation of all degradation products. Also a sufficient run time after the drug peak is to be allowed to obtain the degradants peak eluting after the drug peak.[18] During method development it may happen that the drug peak may hide an impurity or degradant peak that co-elutes with the drug. This requires peak purity analysis which determines the specificity of the method. Direct analysis can be done on line by using photo diode array (PDA) detection. PDA provides information of the homogeneity of the spectral peak but it is not applicable for the degradants that have the similar UV spectrum to the drug. Indirect method involves change in the chromatographic conditions like mobile phase ratio, column, etc. which will affect the peak separation. The spectrum of altered chromatographic condition is then compared with the original spectra. If the degradant peaks and area percentage of the drug peak remains same then it can be confirmed that the drug peak is homogeneous [25]. The degradant that co-elutes with the drug would be acceptable if it is not found to be formed in accelerated and long term storage conditions [26]. The method is then optimized for separating closely eluting peaks by changing flow rate, injection volume, column type and mobile phase ratio.

c. Method validation: The developed SIM is then validated according to USP/ICH guideline for linearity, accuracy, precision, specificity, quantitation limit, detection limit, ruggedness and robustness of the method. It is required to isolate, identify and quantitate the degradants found to be above identification threshold (usually 0.1%). [27,28] If the method does not fall within the acceptance criteria for validation, the method is modified and revalidated

SELECTIVE ANALYTICAL METHODOLOGIES[29-31]: New drug development requires meaningful and reliable analytical data to be produced at various stages of the development.[32-35]

a) Sample set selection for analytical method development
b) Screening of Chromatographic conditions and Phases, typically using the linear solvent-strength model of gradient elution
c) Optimization of the method to fine-tune parameters related to ruggedness and robustness The impurities

can be identified predominantly by following methods;

- Reference standard method
- Spectroscopic method
- Separation method
- Isolation method
- Characterization method

Reference standard method: The key objective of this is to provide clarity to the overall life cycle, qualification and governance of reference standards used in development and control of new drugs. Reference standards serve as the basis of evaluation of both process and product performance and are the benchmarks for assessment of drug safety for patient consumption. These standards are needed, not only for the active ingredients in dosage forms but also for impurities, degradation products, starting materials, process intermediates, and excipients. [18]

Spectroscopic Methods: The following spectroscopic methods can be used:

- Ultraviolet (UV)
- Infrared (IR)
- Nuclear magnetic resonance (NMR)
- Mass spectrometry (MS)

- Ultraviolet (UV): UV at a single wavelength provides minimal selectivity of analysis; however, with the availability of diode array detectors (DAD), it is now possible to get sufficient simultaneous information at various wavelengths to ensure greater selectivity.
- Infrared Spectrophotometry: Infrared spectrophotometry provides specific information on some functional groups that may allow quantification and selectivity. However, low level detection is frequently a problem that may require more involved approaches to circumvent the problem.
- Nuclear Magnetic Resonance Spectroscopy: Nuclear magnetic resonance spectroscopy provides fairly detailed structural information on a molecule and is a very useful method for characterization of impurities; however, it has limited use as a quantitative method because of cost and time considerations.
- Mass Spectrometry: Mass spectrometry provides excellent structural information, and, based on the resolution of the instrument; it may provide an effective differentiating molecules with small differences in molecular weight. However, it has limited use as a quantitative technique because of cost and time considerations.

In summary, IR, NMR, and MS are excellent techniques for characterization of impurities that have been isolated by any of the techniques discussed above. UV has been found to be especially useful for analyzing most samples with high-pressure liquid chromatography. This combination is commonly used in pharmaceutical analysis.

Separation Methods: The following separation methods can be used

- Thin-layer chromatography (TLC)
- Gas chromatography (GC)
- High-pressure liquid chromatography (HPLC)
- Capillary electrophoresis (CE)
- Supercritical fluid chromatography (SFC)

Hyphenated Methods: The following hyphenated methods can be used effectively to monitor impurities.

- GC–MS
- LC–MS
- LC–DAD–MS
- LC–NMR

Isolation methods [36,37]: It is often necessary to isolate impurities because the instrumental methods mentioned above are not available or further confirmation is needed. For example, when hyphenated methods such as LC–MS are not suitable or do not provide unambiguous characterization, it may be necessary to isolate impurities for further confirmation of structure or for conducting toxicity studies.

The following methods have been used for isolation of impurities:

- Solid phase extraction
- Liquid liquid extraction
- Supercritical fluid extraction
- Column chromatography
- Thin-layer chromatography
- Gas chromatography
- High pressure liquid chromatography
- Capillary electrophoresis

Solid Phase Extraction: Strong stage extraction (SPE) is a partition procedure by which intensifies that is disintegrated or suspended in a fluid blend are isolated from different mixes in the blend as per their physical and substance properties. Logical research centers utilize strong stage extraction to focus and sanitize tests for examination. Strong stage extraction can be utilized to disconnect analytes of enthusiasm from a wide assortment of networks, including pee, blood, water, drinks, soil and creature tissue. There are primarily three kinds of SPE.

- Normal stage SPE
- Reverse stage SPE
- Ion trade SPE

Liquid-liquid Extraction: Liquid– fluid extraction, otherwise called dissolvable extraction and dividing, is a technique to isolate mixes dependent on their relative dissolvability's in two distinctive immiscible fluids, typically water and a natural dissolvable. It is an extraction of a substance from one fluid stage into another fluid stage. Liquid– fluid extraction is a fundamental strategy in synthetic research centers, where it is performed utilizing an isolating channel. This kind of process is regularly performed after a concoction response as a major aspect of the work-up.

Segment Chromatography: Segment chromatography in science is a strategy used to sanitize singular substance mixes from blends of mixes. Usually utilized for preparative applications on scales from micrograms up to kilograms. The primary preferred standpoint of segment chromatography is the generally minimal effort and superfluity of the stationary stage utilized all the while. The traditional preparative chromatography section is a glass tube with a measurement from 5 mm to 50 mm and a stature of 5 cm to 1 m with a tap and some sort of a channel (a glass frit or glass fleece plug-to keep the loss of the stationary stage) at the base. Two strategies are commonly used to set up a segment which is the dry strategy and the wet technique.

Thin Layer Chromatography: Thin layer chromatography (TLC) is a chromatography system used to isolate blends. Thin layer chromatography is performed on a sheet of glass, plastic, or aluminum thwart, which is covered with a thin layer of adsorbent material, more often than not silica gel, aluminum oxide, or cellulose (blotting surface paper). This layer of adsorbent is known as the stationary stage. After the example has been connected on the plate, a dissolvable or dissolvable blend (known as the portable stage) is drawn up the plate by means of slim activity. Since various analytes climb the TLC plate at various rates, detachment is accomplished.

Gas Chromatography: Gas chromatography (GC), is a typical kind of chromatography utilized in explanatory science for isolating and breaking down intensifies that can be vaporized without disintegration. Regular employments of GC incorporate testing the immaculateness of a specific substance, or isolating the distinctive segments of a blend (the overall measures of such segments can likewise be resolved). In a few circumstances, GC may help in recognizing a compound. In preparative chromatography, GC can be utilized to plan unadulterated mixes from a blend. In gas chromatography, the portable stage (or "moving stage") is a bearer gas, more often than not a latent gas, for example, helium or an inert gas, for example, nitrogen. The stationary stage is a microscopic instrument used to perform gas chromatography is known as a gas chromatograph.

High Pressure Liquid Chromatography: Superior fluid chromatography (once in a while alluded to as high-weight fluid chromatography), HPLC, is a chromatographic strategy used to isolate a blend of mixes in diagnostic science and organic chemistry with the motivation behind recognizing, evaluating and sanitizing the individual parts of the blend. Some regular precedents are the partition and quantization of execution upgrade drugs (for example steroids) in pee tests, or of nutrient D levels in serum. HPLC commonly uses distinctive sorts of stationary stages (for example sorbents) contained in segments, a siphon that moves the portable stage and test segments through the section, and a finder equipped for giving trademark maintenance times to the example parts and territory tallies mirroring the measure of each analyte going through the identifier. Analyte maintenance time shifts relying upon the quality of its connections with the stationary stage, the structure and stream rate of portable stage utilized, and on the section measurements. HPLC is a type of fluid chromatography that uses little size sections (normally 250 mm or shorter and 4.6 mm i.d. or then again littler; stuffed with littler particles), and higher versatile stage weights contrasted with common fluid chromatography.

CONCLUSION: Constrained debasement investigations of new medication substances and medication items are vital to help create and show particularity of solidness demonstrating strategies and to decide the corruption pathways and debasement items of the dynamic fixings. They were additionally helpful in the examination of the substance and physical steadiness of gem shapes, the stereo chemical security of the medication substance alone and in the medication item and mass-balance issues, and for separating drug substance related debasement items in definitions. The ICH not gave any formal direction. Sufficient debasement required to comprehend the likely degradants for the assessment of soundness demonstrating technique.

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