

**STABILITY INDICATING RP-HPLC: A REVIEW****Jivan Pund\*, Prajwal Tarale, S. G. Jawarkar and Monika Jadhao**

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**\*Corresponding Author****Jivan Pund**Vidyabharti College of  
Pharmacy Amravati.**ABSTRACT**

The top objective of any pharmaceutical industry is to produce products of vital characteristic and great reliably, in a price-effective manner. Improvement of a method is vital for discovery, improvement, and evaluation of drugs within the pharmaceutical formula. The main intention of this evaluation article become to check the improvement and validation of the system employed for the medicine from the beginning of the method to the whole commercial batch of product. On the factor whilst an analytical approach is implemented to produce outcomes for the high-quality of drugs related samples, it is important

that the consequences are reliable. Inside the pharma enterprise, validation coverage is documented for the way to carry out validation, kinds of validation and validation policy are complied with the requirements of desirable production exercise (GMP) guidelines. Validation may be very vital for the effective walking of the pharmaceutical companies. At each level from uncooked fabric to the completed, balance, anywhere validation became carried out. The approach was developed nicely, and validation parameters are defined in phrases of accuracy, specificity, and precision, limit of detection (LOD), limit of quantitation (LOQ), ruggedness, robustness, and machine suitability testing with the instance of positive tablets. All validation parameters are used within the routine and balance analysis.

**KEYWORDS:** Validation, Method development, Limit of quantitation, Limit of detection, Linearity, Robustness, Ruggedness.

**INTRODUCTION**

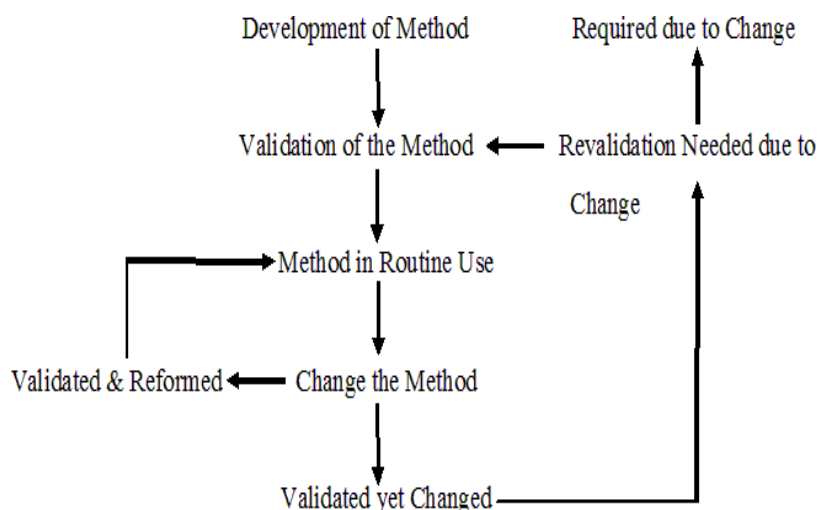
Evaluation is crucial in any product or service, and it's also crucial in drug because it involves lifestyles.<sup>[1]</sup> Analytical chemistry is the evaluation of separation, quantification and chemical additives identification of natural and synthetic materials constituted with one or extra compounds or factors. Analytical chemistry is separated into most important classes, a

qualitative assessment that is to say the identification with reference to the chemical additives exists inside the sample, whereas quantitative assessment estimates the amount of positive detail or compound inside the substance, i.e. the pattern.<sup>[2]</sup> The quantity of drugs introduced into the market is growing every 12 months. Those drugs may also be either new entities or partial structural alteration of the prevailing one. Drug treatments have to be accessible in this sort of kind that excellent in addition to bioavailability, adequate plasma awareness, preferred length, the onset of movement, correct dose, safety, effectiveness and balance on storage of product, might be confident throughout the garage of the products.<sup>[3]</sup> The development of a medication is an extended technique along with drug invention, a studies lab trial, preclinical trying out, clinical testing, and regulatory registration. To moreover improve the adequacy and protection of the medicine after attractiveness, several administrative companies, as an example, the usa meals and drug administration (USFDA) likewise require that the drug product is evaluated for its identification, potency, characteristics, best, balance, and purity earlier than it may be discharged for making use of. For this reason, pharmaceutical validation and procedure controls are crucial in dismissing the problems that is probably encountered.<sup>[4]</sup> Regularly, there's a time lag from the date of creation of a remedy into the marketplace to the date of its attention in pharmacopeias. This takes place because of the possible vulnerabilities within the continuous and greater massive use of these pills, reports of ongoing toxicity (bringing about their withdrawal from the marketplace), development of affected individual resistance and development of higher remedy with the aim of competition. Beneath these situations, requirements and analytical techniques for those drug treatments that might not be approachable in the pharmacopoeias. It will become important, with a view to expand more modern analytical strategies for such medications.<sup>[5]</sup> technique improvement and validation carry out critical features within the discovery, development, and production of medicines. The main purpose of an analytical measure is to get regular, sensible, and correct records. Proven analytical techniques play a great function in achieving this goal. Consequences from methodology validation can be used to select the usual, reliability, and consistency of analytical results, that is associated as an indispensable a part of any realistic analytical exercise. Validation of analytical strategies is also wanted by way of most guidelines and quality standards that effect laboratories.<sup>[6]</sup>

### **Analytical Approach Development**

When there are no definitive strategies are present, new methodologies are being progressed for evaluation of the radical product. To research the presence of either pharmacopoeia or

non-pharmacopoeial product novel strategies are developed to lessen the cost besides time for higher precision and strength. These methodologies are optimized and valid via preliminary runs. Alternate methods are deliberate and vicinity into exercise to change the existing process in the comparative laboratory information with all available deserves and demerits.



**Fig. 1: Life cycle of the analytical method<sup>[7]</sup>**

Drug evaluation famous the identification characterization and backbone of the drugs in combination like dosage paperwork and natural fluids. in some unspecified time in the future of producing method and development of drug the major cause of analytical techniques is to generate facts concerning efficiency (which is probably at once related with the want of a diagnosed dose), impurity (related to protection of the drugs), bioavailability (includes key drug tendencies like crystal type, uniformity of drug and launch of drug), stability(that indicates the degradation product), and effect of manufacturing parameters to confirm that the production of drug product is consistent.

Analyst earlier than the improvement of latest technology, recollect underneath mention standards.

- is that this approach possesses the needful sensitivity?
- is this approach sufficiently selective for direct use without interference by using the other element inside the pattern?
- Is the accuracy and precision possible with this approach?
- Are the reagents and system required in this approach available or acquired at a reasonable fee?
- Is the time requires to perform this approach relevant.<sup>[3]</sup>

➤ **Steps for growing a method**

Various steps are involved inside the improvement of an analytical technique are as follows.

➤ **Characterization of analyte and standard**

- All of the regarded necessary data concerning the analyte and its shape this is to say the bodily and chemical properties inclusive of solubility, optical isomerism, and so forth. Are gathered.
- The standard analyte is identical to 100% purity is obtained. Vital association is to be created for the proper storage (fridge, desiccators, and freezer).
- Within the pattern matrix, whilst more than one parts are to be measured the amount of factors is determined duly imparting the statistics and the accessibility of general are calculated.
- strategies like spectroscopy (UV-visible, FTIR, atomic absorption spectroscopy, etc.), excessive-performance liquid chromatography and gasoline chromatography so on and, are but approximately once coordinated with the steadiness of samples.<sup>[2]</sup>

➤ **Requirement of the method:** Requirement of analytical method is crucial to build up the analytical fig. of gain like linearity, selectivity, specificity, range, accuracy, precision, LOD, LOQ and so on. will be outlined.<sup>[2]</sup>

➤ **Literature survey and earlier strategies:** all of the information of literature associated with the drug are reviewed for its physical and chemical residences, manufacturing, solubility and relevant analytical methods with reference to applicable books, journals, usa pharmacopeia/country wide formulary(USP/NF), association of official agricultural chemists (AOAC) and american society for testing and materials (ASTM) guides and it is extremely convenient to look Chemical Abstracts service automatic automated literature.<sup>[2]</sup>

➤ **Deciding on the technique**

- using the data acquired from the literature, the method is evolving for the reason that method is being modified anywhere wished. now and again, it's far critical to accumulate additional instrumentation to create, modify or replicate and validate existing approaches for analytes and checks.
- If there are not any past suitable ways available to investigate the analyte to be tested.<sup>[2]</sup>

- **Proper instrumentation and preliminary studies:** installation qualification (IQ), operation qualification (OQ), and overall performance qualification (PQ) of tool pertinent to investigate wellknown technique is examined via an appropriate set up of units.<sup>[2]</sup>
- **Optimization:** whilst appearing optimization, once a parameter is modified at a time, and a group of situations are differentiated, earlier than using trial and error technique. This paintings is needed for finished basing on a systematic prepared technique plan duly all important points and documented with relation to useless ends.<sup>[2]</sup>
- Right documentation of analytical fig. of deserves: The proper decided analytical fig. of advantage along with LOD, LOQ, cost, linearity and evaluation time and planning of samples, and so on. Also are recorded.<sup>[2]</sup>
- **Assessment of produced approach with real specimen:** The specimen solution needs to activate unique, whole recognition of the height interest of the medication apart from all one-of-a-kind matrix elements.<sup>[2]</sup>
- **Estimation of percent recuperation of actual samples and demonstration of quantitative sample analysis:** percentage healing of spiked, real trendy medication into a pattern grid which incorporates no analyte is evaluated. Optimization to reproducibility of recuperation from test to check ought to have regarded. It is not continually crucial to get a hundred% recovery so far because the consequences are reproducible to understand with a high level of warranty.<sup>[2]</sup>

## Validation

Validation is a concept that has developed inside the U. S. in 1978. The idea of validation has prolonged for the duration of that time to comprehend an in depth style of sports from analytical processes utilized for the excellent manage of medication to computerized systems for scientific trials, marking or process manage, validation is installed on, however not recommended by way of regulatory specifications and is quality visible as a critical and necessary a part of modern-day precise manufacturing exercise (cGMP).

The phrase validation essentially implies for evaluation of validity or hobby of demonstrating viability. Validation is a staff attempt where it entails humans from diverse departments of the plant. Validation is needed for any new or amended approach to affirm that it's far able to giving consistent and reliable results, once utilized by one-of-a-kind operators using similar instrumentation within the identical or completely extraordinary laboratories.<sup>[8]</sup> Validation is

an important element of fine warranty; it consists of the efficient research of structures, centers, and techniques aimed toward deciding in the event that they execute their deliberate capacities sufficiently and reliably as decided.

### **Validation should on this way be considered in the accompanying situations**

- Absolutely new process.
- Today's equipment.
- Manner and device that have been adjusted to in shape altered wishes and,
- System in which the finished result take a look at is a poor and undependable marker of product first-rate.<sup>[4]</sup>

Crucial degrees in validation

### **The action figuring out with validation studies can be classified mainly into 3 stages.**

#### **Stage 1**

This consists of pre-validation qualification stage which covers all sports figuring out with product studies and development, components pilot batch checking out, scale-up research, trade of innovation to enterprise scale agencies, setting up balance conditions, and managing of in-system, finished pharmaceutical formulations, qualification of device, grasp files, and technique limit.<sup>[4]</sup>

#### **Stage 2**

This involves system validation phase. It's far intended to test that every installed limit of the critical manner parameter is good sized and that first-rate products can be created even beneath the worst situations.<sup>[4]</sup>

#### **Stage 3**

it is also called as the validation upkeep stage, it requires constant evaluate of all system related archives, inclusive of validation of the evaluation reviews, to assure that there were no modifications, departure, screw ups, and alteration to the manufacturing system and that all popular operating techniques (SOPs), concerning exchange manipulate processes, have been discovered. At this segment, the approval crew regarding human beings representing all critical departments also ensures that there have been no modifications/deviations that need to have added approximately requalification and revalidation.<sup>[4]</sup>

## Types of validation

Validation is classified into following types.

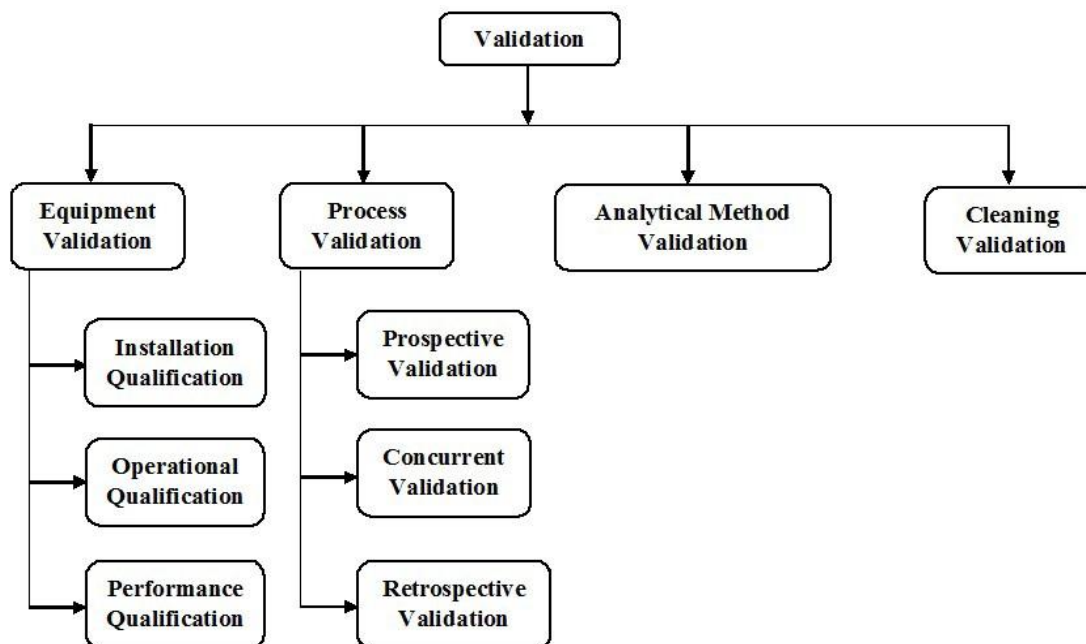


Fig. 2: Validation types<sup>[9]</sup>

## Equipment validation

➤ The key concept of validation is to give a high degree of pronounced affirmation that the system and the process agree to the written tips. The diploma (or intensity) is dictated by means of the complexity of the tool or device. The validation should provide the crucial records and test strategies required to offer that the device and technique meet determined prerequisites. gadget Validation consists of the following.

➤ **Installation qualification (IQ):** IQ guarantees all vital processing, packaging system, and ancillary items are in compliance with the set up. It checks that the device has been set up or hooked up as in step with the producer's idea in a systematic way and placed in surrounding appropriate for its meant purpose.<sup>[10]</sup>

➤ Installation qualification points include

- Device layout individual this is the cloth of production cleanability and plenty of others.
- Installation situations like wiring, capability, software and so on.
- Calibration, preventative safety, cleansing plans.
- Protection characteristics.
- Supplier documentation, prints, illustrations, and hand operated.
- Software documentation.
- Enlist the spare additives.

- Environment-associated situations like easy room necessities, humidity, and temperature.<sup>[11]</sup>

➤ **Operational qualification (OQ):** OQ carried out to present a high degree of diploma of affirmation that the device works as proposed.<sup>[10]</sup>

#### **OQ concerns consist of**

- Process control limits like temperature, time, and stress, line velocity, set up conditions, and so on.
- Software parameters.
- Crude material details.
- Process operating methods.
- Material managing necessities.
- Process change control.
- Training.
- Short-term balance and capability of the technique.

#### **Necessity for cleaning validation**

To check the viability of cleaning techniques and to make sure that no risks are related to cross-contamination of API or detergents.<sup>[16,17]</sup>

#### **Cleaning validation protocol**

- The goal of the validation procedure.
- Obligations regarding performing and endorsing the validation study.
- Equipment details.
- The interval between the end of production and the start of the cleaning techniques.
- Cleaning methods to be utilized for every product, each manufacturing device or each piece of equipment.
- The quantity of the cleaning cycle to be performed continuously.
- Routine checking equipment.
- Sampling techniques, including the basis for why a specific sampling technique is utilized.
- Clearly defined sampling areas.
- Information on recovery studies, where suitable.
- Analytical techniques including LOD and LOQ.



The acceptance criteria, along with including the method of reasoning for setting specified limits.<sup>[18]</sup>

### Importance of validation

- Assured high quality.
- Time boundation.
- Optimization of the method.
- Minimum batch product failure, enhanced efficiency, manufacturing, and productivity.
- Quality cost decreased.
- Rejection decreased.
- Yield increases.
- Fewer complaints about process related issues.
- Fast and realistic start-up of new equipment's.
- Increased worker consciousness of the process.<sup>[9]</sup>

### Validation parameters

The principle purpose of method validation is to provide proof that the technique will what it is meant to do, accurately, reliable and consistent.<sup>[9]</sup> The validation parameters as consistent with ICH tips are described beneath.

**Accuracy:** Accuracy is expressed because the nearness of agreement among the values observed and values which might be already available. It could additionally be defined as the closeness between the proper cost and the found fee. it's miles from time to time known as trueness, and it is able to be decided with the aid of the use of as a minimum nine determinations over at least three attention over the required range.<sup>[19]</sup>

Accuracy of prochlorperazine maleate (seasoned) and betahistine hydrochloride (bet) was studied with the aid of the usual addition method at three specific tiers (50%, 100%, and 120%). A recognised quantity of drug changed into introduced to the pre-analysed sample and percentage healing calculated. while this approach was used for accuracy, the restoration changed into found to be 99.32% for betahistine hydrochloride and 99% for prochlorperazine maleate.<sup>[20]</sup>

For the concurrent dedication of nitazoxanide and ofloxacin accuracy changed into studied by means of the usual addition approach at 5 different stages (50%, 75%, 100%, a 125%, and 150%). The effects imply that the recoveries had been found to be within the variety of 80% to 120%, therefore, the method is accurate.<sup>[21]</sup>

**Precision:** The exactness of an analytical system expresses the nearness of settlement (degree of scatter) between a collections of measurements obtained from unique sampling of a uniform pattern under the prescribed situations.<sup>[23]</sup>

Precision can be taken into consideration at three levels:

- **Repeatability:** It expresses the exactness below a similar operating circumstance over a brief c programming language of time and also referred as intra-assay precision. Not less than six replicates take a look at instruction of a comparable or regular pattern prepared at the 100% test.<sup>[24]</sup>
- **Intermediate precision:** It expresses the exactness under internal research laboratories, in wonderful days, through wonderful analyst, on awesome contraptions/device. Special analysts every getting ready six pattern solutions, as according to distinctive method.<sup>[25]</sup>
- **Reproducibility:** It refers to the precision among exceptional analytical labs. Each studies facility installation an aggregate of six sample answers, in keeping with the analytical technique.<sup>[24]</sup>

The precision of prochlorperazine maleate (pro) and betahistine hydrochloride (bet) method turned into determined with the aid of interday and intraday version (% RSD). Intra-day precision turned into achieved via studying wellknown drug answers in the calibration variety, three instances on the equal day. Inter-day precision turned into finished with the aid of analysing drug answers inside the calibration range on 3 special days over a period of 7 days. The low % RSD values of interday (1.02 to 1.48% for bet at 252.9 nm and 0.68 to 0.82% for pro at 260.15 nm) and intraday (0.75 to 0.09% for bet at 252.9 nm and 0.27 to 0.61% for seasoned at 260.15 nm variation for guess and seasoned, found out that the approach is precise.<sup>[20]</sup>

**Specificity:** For each degree of improvement, the analytical technique should display specificity. The technique turned into need to have the energy to unequivocally verify the analyte of interest while in the presence of all expected elements, which could embody degradants, excipients/sample matrix, and sample clean peaks.<sup>[26]</sup>

Specificity turned into carried out to determine the retention time of each drug in a combination and in the sample. The retention time of standard capsules in my view turned into decided, and it became determined to be 3.750 min and 1.533 min for nitazoxanide and ofloxacin and retention time of both capsules in the wellknown mix changed into discovered to be 3.760 min for nitazoxanide and 1.542 min for ofloxacin respectively.<sup>[21]</sup>

**Limit of detection (LOD):** Lowest amount of an analyte which may be detected by the chromatographical separation however it isn't always essential that this amount will quantify as a particular price. A clean decision is injected and top to height quantitative noise relation we should calculate from blank chromatograms. Then, calculate the attention at the signal to quantitative noise relation is regarding 3:1. LOD may be expressed as  $LOD = 3.3SD/S$  Wherein, SD = trendy deviation of response, S = Slope of calibration curve.<sup>[27]</sup>

The LOD fee of betahistine hydrochloride (wager) and prochlorperazine maleate (pro) is zero.29µg/ml and 0.34µg/ml respectively.<sup>[20]</sup>

LOD of the paracetamol became studied by means of the signal to noise ratio, and the result become discovered to be 120µg/ml.<sup>[22]</sup>

**Limit of Quantitation (LOQ):** it is characterised by the least quantity of an analyte that can be quantified with exactness and precision.

LOQ can be communicated as  $LOQ = 10SD/S$

Where SD = wellknown deviation of reaction, S = Slope of calibration curve.<sup>[28]</sup>

The LOQ cost of betahistine hydrochloride (bet) and prochlorperazine maleate (pro) is 0.957µg/ml and 1.12µg/ml respectively.<sup>[20]</sup>

LOQ of the paracetamol was studied by way of the sign to noise ratio, and the end result become determined to be 360µg/ml.<sup>[22]</sup>

A few normal techniques, strategies for the assessment of LOD and LOQ are as follows.

- Visual inspection,
- Sign to noise ratio,
- Fashionable deviation of the clean, and
- Regression line at low concentrations.<sup>[29]</sup>

**Linearity:** Linearity may be characterised because the capacity of an analytical method to produce consequences that are without delay related to the attention of an analyte in the.<sup>[30]</sup> widespread answer of betahistine hydrochloride (guess) and prochlorperazine maleate

(seasoned) become taken in a 10 ml volumetric flask and diluted with 0.1 N HCL to get the final concentration within the range of four to 24µg/ml for wayer and 3 to 18µg/ml for seasoned. Prepared six times in this calibration range and absorbance determined on the respective wavelength for every drug alone. The outcomes show appropriate linearity among absorbance and attention within the prescribed attention range for each the medicine.<sup>[20]</sup>

For simultaneous estimation of nitazoxanide and ofloxacin linearity carry out as 5 distinct concentrations of fashionable mixtures prepared, 50%, seventy five%, 100%, a 125%, 150% have been injected, and chromatogram turned into recorded. The correlation coefficient become calculated and become determined to be more than 0.ninety nine for both the medicine which might be in the limit.<sup>[21]</sup>

The linearity of paracetamol was carried out by means of making ready one-of-a-kind concentrations (6.25, 12.5, 25, 50, and a 1000µg/ml) from a stock solution of 10 mg/ml. the answer of 20 µl was injected into column 3 instances. Linearity of paracetamol became found within the attention variety of 6.25-100µg/ml with a correlation coefficient of 0.999.<sup>[22]</sup>

**Range:** it can be characterised as the c program languageperiod among upper and decrease portions of analyte in the sample. Minimum of the required range to be 80% to 120% of the check pattern for the assay take a look at.<sup>[31]</sup>

**Ruggedness:** Ruggedness is the diploma or measure of reproducibility under special situations which include in distinct laboratories, special analyst, distinct machines, environmental conditions, operators and many others.<sup>[32]</sup>

Inside the simultaneous estimation of nitazoxanide and ofloxacin, ruggedness became accomplished by way of exceptional analyst and in extraordinary laboratories in one-of-a-kind days to checks for any version inside the chromatography. The % RSD for region and retention time was calculated for dedication.<sup>[21]</sup>

**Robustness:** it's far characterised by the level of capability of an analytical approach, to live comparable by way of minute purposely change inside the method parameter. The one of a kind approach parameters which may be changed in excessive-overall performance liquid chromatography are pH, flow price, the temperature of the column and mobile section composition.<sup>[33]</sup>

In the simultaneous estimation of nitazoxanide and ofloxacin, the robustness of the proposed approach validated by using to carry out analysis below variable flow rates. The flow rate as consistent with the evolved approach is 1.5 ml/min. moderate change in flow rate is 1.3 ml/min and 1.7 ml/min and chromatogram recorded. Because of a moderate change within the flow rate of approach shows accurate results and continue to be unaffected by means of that minute exchange. So, we can say that the method is robust.<sup>[21]</sup>

For robustness of paracetamol, small modification in the flow rate, % of acetonitrile and pH of the mobile phase, the approach remains unaffected and % RSD value received are approximately close to.<sup>[22]</sup>

**System suitability parameters:** system suitability test is used to test the sensitivity, resolution, and reproducibility of the chromatographic machine are well for the analysis to be accomplished. The factors in particular utilized in system suitability are tailing component, some of the theoretical plate, retention time, resolution, and so etc.<sup>[34,38]</sup>

#### FORCED DEGRADATION STUDY

The ICH guideline Q1A, exemplifies the core stability recommendations and testing of the features which are susceptible to change during storage, that are likely to influence the quality, safety and efficacy must be done by validated SIM. It is also mentioned that forced decomposition studies at temperatures in 10°C increments above the accelerated temperatures, extremes pH and under oxidative and photolytic conditions should be carried out on the drug substance, so as to establish the inherent stability characteristics and degradation pathways to support the suitability of the proposed analytical procedures.<sup>[39]</sup>

The ICH Q1B, outlines the photostability testing should be an integral part of stress studies. Generally, an ICH dose of 1.2×10<sup>6</sup> and 2.4×10<sup>6</sup> lxh of fluorescent light and 200 Wh/m<sup>2</sup> UV light is recommended to estimate the photostability considerations of the given drug substance and products.

The ICH guideline Q3B emphasizes on providing documented evidence that analytical procedures are validated and suitable for the detection and quantitation of degradation products. The guideline gives a clear concept on the threshold levels of degradation products in drug substances, in terms of reporting, identification and quantitation thresholds.

The ICH guideline Q6A provides note for guidance on specifications and mentions the

requirement of stability- indicating assays under Universal Tests/Criteria for drug substances and drug products. The similar requirement has been laid down in the ICH Q5C guideline on Stability Testing of Biotechnological/Biological Products. Such products have distinguishing characteristics and thus the document outlines the necessity of determining the changes in identity, purity and potency of the product.<sup>[40-43]</sup>

### **Aim and Objective of Forced Degradation Study**

- i. To determine drug substances and drug products degradation pathways.
- ii. To illuminate the structure of degradation products.
- iii. To differentiate degradation product that are related to drug product from product in formulation.
- iv. To reveal the degradation mechanisms such as hydrolysis, oxidation, thermolysis or photolysis of the drug substance and drug product.<sup>3-4</sup>
- v. To understand the chemical properties of drug molecule
- vi. To produce stable formulation.
- vii. To produce a degradation profile similar to that of what would be observed in a formal stability study under ICH conditions.<sup>[44-46]</sup>

### **When carry out forced degradation<sup>[45-48]</sup>**

It is very prime to know when to perform forced degradation studies for the development of new drug substance and new drug product. FDA guidance states that stress testing should be performed in phase III of regulatory submission process. 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. These stress studies are conducted on a single batch. The results should be summarized and capitulate in an annual report. However, starting stress testing early in preclinical phase or phase I of clinical trials is highly vitalize and should be conducted on drug substance to obtain sufficient time for identifying degradation products and structure elucidation as well as optimizing the stress conditions. An early stress study also gives timely recommendations for making changes for better in the manufacturing.<sup>[10]</sup>

### **Most common stress conditions used in Forced degradation study**

The following are general conditions that should be considered when conducting forced degradation studies.

### Hydrolytic degradation

Hydrolysis is a chemical process that includes decomposition of a chemical compound by reaction with water. Under acidic and basic conditions hydrolytic study includes catalysis of ionisable functional groups present in the molecule. Acid or base stress testing involves forced degradation of a drug substance by exposure to acidic or basic conditions which generates primary degradants in desirable range. On the stability of the drug substance the selection of the type and concentrations of acid or base depends. Sodium hydroxide or potassium hydroxide (0.1–1M) for base hydrolysis and Hydrochloric acid or sulphuric acids for acid hydrolysis is suggested as suitable reagents for hydrolysis. The hydrolytic degradation of a new drug in acidic and alkaline condition can be studied by refluxing the drug in 0.1 N HCl or 0.1 N NaOH. If reasonable degradation is seen, testing can be stopped at this point. However in case no degradation is seen under these conditions the drug should be refluxed in acid/alkali of higher strength & for longer duration of time. Alternatively if total degradation is seen after subjecting the drugs to initial condition, acid/alkali strength can be decreased along with decrease in reaction temperature.

### Photolytic conditions

The photo stability testing of drug substances must be evaluated to demonstrate that a light exposure does not result in unacceptable change. Photo stability studies are performed to generate primary degradants of drug substance by exposure to UV or fluorescent conditions. Some recommended conditions for photostability testing are described in ICH guidelines. Samples of drug substance and solid/liquid drug product should be exposed to a minimum of 1.2million lx h and 200 W h/m<sup>2</sup> light. The most commonly accepted wavelength of light is in the range of 300– 800 nm to cause the photolytic degradation. The maximum illumination recommended is 6 million lx h. Light stress conditions can induce photo oxidation by free radical mechanism. Functional groups like carbonyls, nitroaromatic, Noxide, alkenes,aryl chlorides, weak C–H and O–H bonds, sulphides and polyenes are likely to introduce drug photosensitivity.

### Oxidation Conditions

Hydrogen peroxide is broadly used for oxidation of drug substances in forced degradation studies. Other oxidizing agents such as metal ions, oxygen, and radical initiator (e.g., azobisisobutyronitrile, AIBN) can additionally be used. Choice of an oxidizing agent, its concentration, and process depends on the drug substance. It is reported that subjecting the

solutions to 0.1–3% hydrogen peroxide at neutral pH and room temperature for seven days or up to a maximum 20% degradation could potentially produce relevant degradation consequences. Oxidative degradation of drug substance and drug products forms reactive anions and cations. Amines, sulphides and phenols are capable to electron transfer oxidation to give N-oxides, hydroxylamine, sulfones and sulfoxide. The functional groups with labile hydrogen like benzylic carbon, allylic carbon, and tertiary carbon or  $\alpha$ -positions with respect to hetero atom to oxidation to form hydro peroxides, hydroxide ketone.

### Thermal Conditions

The thermal degradation study shall be carried out at more intense conditions than proposed ICH Q1A accelerated test conditions. Solid-state drug product and drug substances samples should be exposed to dry and wet heat, while liquid drug products should be exposed to dry heat. Thermal Studies may be managed at higher temperatures for a shorter period. Effect of temperature on thermal degradation of a substance is studied through the Arrhenius equation.

$$k = Ae^{-E_a/RT}$$

Where,  $k$  is specific reaction rate,  $E_a$  is energy of activation,  $A$  is frequency factor,  $R$  is gas constant (1.987 cal/deg mole) and  $T$  is absolute temperature Thermal degradation study is carried out at 40–80°C.

**Table 2: Conditions used mainly for forced degradation studies.**

Degradation Type	Experimental Conditions	Storage Conditions	Sampling Time (Days)
Hydrolysis	Control API9(No Acid or Base)	40°C, 60°C	1,3,5
	0.1M HCl	40°C, 60°C	1,3,5
	0.1 M NaOH	40°C, 60°C	1,3,5
	Acid Control	40°C, 60°C	1,3,5
	Base Control	40°C, 60°C	1,3,5
	pH:2,4,6,8	40°C, 60°C	1,3,5
Oxidation	3% H <sub>2</sub> O <sub>2</sub>	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 1x ICH	NA	1,3,5
	Light 3x ICH	NA	1,3,5
	Light Control	NA	1,3,5
Thermal	Thermal	60°C	1,3,5
	Heat Chamber	60°C/75%RH	1,3,5
	Heat Chamber	H	1,3,5
	Heat Chamber Heat Control	60°C	1,3,5



		80 <sup>0</sup> C/75%R H 80 <sup>0</sup> C Room Temp.	1,3,5
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## CONCLUSION

This newsletter offers a concept that what's validation, its kinds, why it's miles vital, how to expand a method and how to carry out the validation technique to illustrate that the approach is in a position for its proposed reason. All validation parameters inclusive of linearity, LOQ, LOD, variety, specificity, robustness, ruggedness and system suitability are described well with examples of sure pills. Forced degradation studies provide information on possible degradation products and degradation pathways of the active ingredients and help to elucidate the structure of the degradants and impurities. The products formed from forced degradation studies are potential degradation products that may or may not be formed under relevant storage conditions but which help in the development of the stability indication method. It is better to start degradation studies earlier in the drug development process to have enough time to get more information about the stability of the molecule. This information in turn will help improve the manufacturing process of the formulation and determine the storage conditions. Since no specific set of conditions is applicable to all pharmaceutical products and pharmaceutical substances, and the normative guide does not specify the conditions that will be used, this study requires the experimenter to use common sense. The objective of any strategy used for forced degradation is to produce the desired amount of degradation, that is, 5-20%. Validation is an essential method inside the pharma branch, and it's far used to assure that the exceptional is worked into the techniques supporting the improvement of drug and production.

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