DEVELOPMENT OF STABILITY INDICATING ASSAY METHOD: A REVIEW

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**Keywords:** Stability testing, Stability indicating methods, Regulatory requirements, Drug substance, Drug product, Shelf life

**ABSTRACT:** Stability of pharmaceuticals is defined as the ability to retain the quality, purity, identity, and safety throughout the shelf life of products. It is of main concern because drug substance and products lose its Potency and quality after the time passes, due to change in environmental conditions such as temperature, light, and humidity. It is essential that quality of drugs should maintain throw out the life cycle of drug products for their safe and effective use. The need for constant monitoring of the drug substance and product for their quality and purity has the origin of the development of various stability testing methods. Stability testing now becomes a regulatory requirement for filing NDA and ANDA to USFDA and various regulatory agencies. Stability indicating methods had originated from advancement in various analytical instrument technologies. Stability indicating methods of drug substance and products have the ability for separation, identification, qualification, and quantification of all impurities associated with drug substance and drug product at any storage Conditions to give the exact concentration of drug substance or analyte at any time point over the shelf life of products and beyond. These are helpful to understand the degradation pathways as well as obtaining knowledge about impurities developed during processing which should not be present in drug products or have a specific limit, if present. The present review explored the importance, regulatory requirement, various analytical techniques used and some successfully developed stability indicating methods for different drug substance and drug products.

**INTRODUCTION:** Drug substance is defined as an active ingredient which is used in the treatment and mitigation of various diseases and disorders whereas, drug products are defined as the different marketed formulations present for drug substance to take or easily administered in the patient for showing its required therapeutic effect.

Both these required to go through the extensive study of its stability to estimate its shelf life and re-test period. Balance of drug substance and products is nothing but the ability of it to retain its identity, quality, purity during its life cycle with given storage conditions ¹.

Earlier stability indicating methods had the main focus is given on the estimation of the amount of drug substance present in the marketed preparations. So, such type of testing methods is known as specific stability testing methods. These methods involve the estimation of the amount of drug substance in the presence of other impurities, excipients, degradation products without their separations.
The presence of other impurities and degradation products of drug substance may be responsible for the adverse effects of drug products. It makes the given drug product unsafe to use clinically.

There becomes a requirement to study all impurities associated with drugs and their degradation products thoroughly, along with the normal assay test of drug substance at any given time point. The selective stability testing methods can separate, identify, and quantify the amount of drug concentration along with its degradation products and process impurities.

USFDA strongly recommends stability testing because some incidence happened in the past which shows the impurities associated with drug shows severe toxic effects, which causes death also. Whenever it is required to produce safe and effective drug products for human use, there is great need of maintains its purity and quality of drug substance at any storage conditions. There should not present any impurity at all in drugs products and if present, should have specific limits such that it should not be responsible for to produce any kind of adverse effects of drug products.

ICH guidelines have guided the preparation of stability testing protocols for registration of new drug substance and product in USA, Japan, and European Union countries. It provides the requirement of three types of stability testing such as long term stability studies, accelerated stability study and intermediate stability study for four climatic zones. ICH guidelines also guide qualification, identification, and quantification of impurities in drug substances and drug products to determining its thresholds.

Stability indicating methods are developed to determine the ability of drug stability, i.e. drug can retain its purity, quality, identity drug substance, and drug product underexpose of verities of storage conditions such as temperature, humidity, light. There is three-way of degradation of drug products that are hydrolysis, oxidation, thermal degradation of drugs.

**Regulatory Requirement of Stability Indicating Method Development and Validation:** Code for federal regulations CFR 211.16 (a) recommends that there should be written protocol for study the drug product stability. CFR 211.137 (a) states that there should be expiration dates bear on the drug product label by evaluating their stability test data. CFR 314.50 (d) (1) (i) states that there should be full study work carried out to determine its chemical and physical stability. CFR 314.50 (d) (1) (ii) indicates that to determine expiration date by using obtained stability data.

CFR 312.23 (a) (7) (iv) (a) states that to obtain information which is sufficient to support the stability of the drug substance for the study of the toxicology and the planned clinical studies. CFR 312.23 (a) (7) (iv) (b) states that the requirement of information available to assure the product’s stability for proceeding to clinical studies. 21 CFR 312.23 (a) (7) (ii) recommends that stability data are required in all phases of the IND to demonstrate that the DS and DP are retained their quality and purity during proposed clinical investigation.

International Conference on Harmonization (ICH) is an expert working group to harmonize the technical, regulatory requirement for registration of products of human use in Japan, USA, and EU. WHO, ICH Q1A (R2), ICH Q1B, ICHQ1C, ICHQ1D, and ICH Q1E are the regulatory guidance given for preparing stability data testing protocols, report generation, and evaluation of stability data. Furthermore, ICH Q3, ICH Q6, and ICH Q7 also include supportive stability data required for registration of drug substance and drug products.

**TABLE 1: REGULATORY GUIDELINE REFERENCES RELATED TO STABILITY TESTING**

<table>
<thead>
<tr>
<th>Regulatory guidelines</th>
<th>Implemented for</th>
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<tbody>
<tr>
<td>ICH Q1A(R2)</td>
<td>Stability testing of new drug substance and drug products</td>
</tr>
<tr>
<td>ICH Q1B</td>
<td>Photo-Stability testing of new drug substance and drug products</td>
</tr>
<tr>
<td>ICH Q1C</td>
<td>Stability testing of the new dosage form</td>
</tr>
<tr>
<td>ICH Q1D</td>
<td>Bracketing and matrixing study of stability testing</td>
</tr>
<tr>
<td>ICH Q1E</td>
<td>Evaluation of stability data</td>
</tr>
<tr>
<td>ICH Q2R1</td>
<td>Validation of analytical procedures: Text and methodology</td>
</tr>
<tr>
<td>ICH Q3A R2</td>
<td>Impurities in drug substance</td>
</tr>
<tr>
<td>ICH Q3B R2</td>
<td>Impurities in drug product</td>
</tr>
<tr>
<td>ICH Q6</td>
<td>Specifications for a test of acceptance</td>
</tr>
<tr>
<td>ICH Q7</td>
<td>CGM practices for manufacturing API</td>
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</tbody>
</table>
CDSCO is an organization which provides guidance to file NDA and ANDA application within the territory of India. Stability study can be started with a Phase I clinical trial. The stability study work should be complete before phase III of a clinical trial. In the case of BE study, the stability testing should be finished up to the level of phase III of clinical trial 24.

**Preparing Stability Testing Protocols:** Stability testing is carried out in two ways long term stability studies, and short term accelerated stability testing. If any significant change in stability is observed during accelerated stability testing, then intermediate stability study will be carried out. These testing should be carried out on the first three production batches 14.

In long term stability studies the four-point testing is carried out (0, 3, 6, 9, 12) quarterly for the first year, biannually in the second year and then annually up to the shelf life of that product. Whereas in accelerated stability testing three-point study (0, 03, 06) is carried out if a significant change in stability observed then the intermediate study is carried out for additional six months (0, 3, 6, 09, 12) 14.

**TABLE 2: STABILITY TESTING CONDITIONS**

<table>
<thead>
<tr>
<th>Stability study</th>
<th>Environment conditions</th>
<th>Period of study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long term study</td>
<td>20 °C ± 2 °C / 60% ± 5%</td>
<td>12 month (Quarterly)</td>
</tr>
<tr>
<td>or</td>
<td>or</td>
<td></td>
</tr>
<tr>
<td>or</td>
<td>30 °C ± 2 °C / 65% ± 5%</td>
<td></td>
</tr>
<tr>
<td>Accelerated</td>
<td>40 °C ± 2 °C / 75% ± 5%</td>
<td>06 month (0, 03, 06)</td>
</tr>
<tr>
<td>stability study</td>
<td>Intermediate</td>
<td></td>
</tr>
<tr>
<td>or</td>
<td>30 °C ± 2 °C / 65% ± 5%</td>
<td>06 Month (06, 09,12)</td>
</tr>
<tr>
<td>or</td>
<td>or</td>
<td></td>
</tr>
<tr>
<td>or</td>
<td>30 °C ± 2 °C / 65% ± 5%</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 3: DRUG EXAMPLES WITH ANALYTICAL INSTRUMENT USED FOR STABILITY STUDIES**

<table>
<thead>
<tr>
<th>Drug examples</th>
<th>Analytical instrument used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albendazole, Atazanavir Sulfate, Desloratadine, Cefexime &amp; dicloxacilline,</td>
<td>HPLC-UV SIM 32-42</td>
</tr>
<tr>
<td>Temozolamide, Letrozol, Praziquantel, Prulifloxacin, Buprinorhine HCl and</td>
<td></td>
</tr>
<tr>
<td>Nalaxone HCl, Guaifenesin &amp; pseudoephedrine, RizatRIPTAN Benzoate, Doxorubicin,</td>
<td></td>
</tr>
<tr>
<td>Rufinamide, Rolifimlart, Pragabalin, Nizatidine, Naftopidil, Dexamethasone and</td>
<td></td>
</tr>
<tr>
<td>Moxifloxacin, Levocabastine, AMLO-VAL-HCTZ, Eremamtholide C, Silymerin and</td>
<td></td>
</tr>
<tr>
<td>curcumin, Sofosbuvir and Ledipasvir, n-acetyl cysteine, Diclofenac, Piracetam,</td>
<td></td>
</tr>
<tr>
<td>Rivaroxaban, Ofloxacine &amp; ornidazole, Isoflavone aglycone in soybean,</td>
<td></td>
</tr>
<tr>
<td>Desonide, Loratadine, Clobetasol, Nicardipine, Azilsartan medoxomil Pottassium,</td>
<td></td>
</tr>
<tr>
<td>Ezetimibe, Simavastatin, Zidovudine</td>
<td></td>
</tr>
</tbody>
</table>

Stress testing is also recommended by ICH guidelines but in a nonspecific way, an *i.e.* increment of 10 °C after accelerated stability testing conditions (50°, 60°, 70°) and humidity (75% and More) to susceptible the drug product for hydrolysis, oxidative degradation. Photostability study is also carried out in stress testing for possibilities of photodegradation of drug products. Degradation of a drug product under the influence of pH renders the study of acid and base hydrolysis of drug which also came under stress testing. Stability indicating assay methods is useful to study the possible degradation pathway of drug substance and drug products. Forced degradation studies are recommended to study the degradation pathway and knowledge of degrading products of drug substance and their products 25-29.

**Analytical Instrument used in Stability Indicating Method Development:** The advancement in analytical instrument techniques makes it easier to develop the SIM. It should have good separation between the drug substance, its impurities, and degradant products. It should be posse’s high sensitivity towards analyzing of minimum concentration of drug substance. The TLC, HPLC-DAD, HPLC-UV, HPTLC, HPLC-MS, LC-MS/MS, LC-NMR, these are some techniques that have high sensitivity and resolution power to develop the effective stability indicating method 30. TLC method has advantages over HPLC is the volume of mobile phase required is small, large no. of the sample can be analyzed in one single plate by densitometry method. HPTLC has higher sensitivity than TLC but less sensitive than HPLC.

HPTLC has advantages over large no of the sample can apply on a single plate, and the amount of mobile phase required is small, so it has cost-effective analysis. HPLC UV method is the most
commonly used method for development of stability indicating method, but it has a limit of its detection ability; however it is more sensitive than TLC and HPTLC method. HPLC-PDA or DAD has a wide range of detection. One can determine the wavelength where all impurities, degradant products, and drug substance show absorbance so, it makes easy detection, separation, and quantification of all contaminants, related substances to give exact drug concentration at any time point during its storage.

HPLC-MS has higher sensitivity to analyze the small quantity of analyte also. In such a way the HPLC-MS/MS shows to study the fate of a drug in human biological fluids, i.e. drug plasma concentration level. LC-NMR is also a highly sensitive technique to have the ability of separation of enantiomers in which one of them considered as an impurity of drug substance.  

**How to Approach Stability Indicating Method Development and Validation:**  
**Sample Generation:** Suitable sample preparation is important to step in SIAM. Knowing of its drug chemistry, its degradation pathway is essential for the selection of the method. Prepared sample undergoes stress testing so prior sample information of its related products, degradation profile; associated impurities will help in developing effective SIM.  

**Method Selection:** Method selection depends on its specificity and selectivity; the sensitivity of methods to analyze the given sample. Method selection is based on an extensive literature survey for such likely sample on which methods had already developed in the past. It depends on the ability of method to separate the drug API and its degraded product, associated impurities.  

**Method Development:** Method development is carried out by choosing the specific column and selecting a mobile phase which can efficiently separate the active constituent and its related product. Method development is may be a trial and error method to optimize the parameters to yield effective separation.  

**Method Validation:** Method validation is done as per ICH guidelines. Accuracy, precision, linearity, LOQ, LOD, robustness, and ruggedness are the parameters tested on each novel method developed. RSD value should be less than 2% as per ICH guideline.  

**CONCLUSION:** Stability indicating assay methods now become a regulatory requirement by the FDA because it is related to the safety of drug product. With the development of chromatographic techniques, analytical method development and validation gain importance in recent days because of having high sensitivity, selectivity and have the ability to separate the analyte of interest and its related substances which is useful to develop its limits of specifications in finished products.

Conventional testing of the specific analyte of interest is get converted into modern testing of the selective analyte of interest with studying its associated impurities and related products developed during its storage conditions such that one can able to determine exact and precise concentration of the analyte at any time point during its storage. It is required to develop and validate the method to ensure the stability of drug substance and drug products up to its retest period and shelf life of the finished product. Furthermore, it involves the stress testing to know the degradation pathway and stating its limits of specification present in the finished product during its shelf life which results in safe & effective use of the product.  

**ACKNOWLEDGEMENT:** The authors are thankful to the School of Pharmacy, Swami Ramanand Teerth Marathwada University, Nanded for support and encouragement.  

**CONFLICT OF INTEREST:** The authors declared no conflicts of interest.  

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How to cite this article: