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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPLC METHOD: A REVIEW

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ABSTRACT

A sensitive, precise, specific, linear and stability indicating HPLC method was developed for the analysis of drug product stability. HPLC method should be able to separate, detect and quantify the various drug related degradant that can form on storage or manufacturing and quantify any drug related impurity that may be introduced during synthesis and also for stability studies in view of its capability to separate degradation products. This article discusses the strategies and the issue pertinent to designing stability indicating HPLC method for drug substance. It further understanding of the chemistry of the drug substance and facilitates development of stability indicating methodology. In this review article also discussed about the strategy and importance of validation of analytical methods. The method was validated as per ICH guidelines.

Key Words: Stability indicating method, HPLC, Validation

INTRODUCTION

The stability-indicating assay is a method that is employed for the analysis of stability samples in pharmaceutical industry. With the advent of International Conference on Harmonization (ICH) guidelines, the requirement of establishment of stability-indicating assay method (SIAM) has become more clearly mandated. The guidelines explicitly require conduct of forced decomposition studies under a variety of conditions, like pH, light, oxidation, dry heat, etc. and separation of drug from degradation products. The method is expected to allow analysis of individual degradation products. According to FDA guidance document a stability-indicating method is "a validated quantitative analytical procedure that can detect the changes with time in the pertinent properties of the drug substance and drug product. A stability-indicating method

accurately measures the active ingredients without interference from degradation products, process impurities, excipients or other potential impurities. Accordingly, the purpose of this write-up is to suggest a systematic approach for the development of validated SIAMs that should meet the current ICH and regulatory requirements. The discussion also touches upon various critical issues such as the extent of separation of degradation products, establishment of mass balance etc. which are important with respect to the development of stability-indicating assays, but are not yet fully resolved. Some other aspects like suitability of pharmacopoeial methods for the purpose and the role of SIAMs in stability evaluation of biological/biotechnological substances and products are also delved upon. *[1]*

STABILITY INDICATING ANALYTICAL METHOD

It is a validated quantitative analytical method that can detect the change with time in the chemical, physical or microbiological properties of the drug substance and drug product and that are specific so that the content of active ingredient, degradation can be accurately measured without interference. The purpose of stability testing is to provide evidence on how the quality of an active substance or Pharmaceutical product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light. In addition, product-related factors influence the stability, for example the chemical and physical properties of the active substance and the Pharmaceutical excipients, the dosage form and its composition, the manufacturing process, the nature of the container-closure system and the properties of the packaging materials. Also, the stability of excipients that may contain or form reactive degradation products, have to be considered. *[2]*

Regulatory Status of Stability-Indicating Assays

The ICH guidelines have been incorporated as law in the EU, Japan and in the US, but in reality, besides these other countries are also using them. As these guidelines reflect the current inspectional tendencies, they carry the de facto force of regulation. The ICH guideline Q1A on Stability Testing of New Drug Substances and Products emphasizes that the testing of those features which are susceptible to change during storage and are likely to influence quality, safety and efficacy must be done by validated stability-indicating testing methods. It is also mentioned that forced decomposition studies (stress testing) at temperatures in 10 °C increments above the accelerated temperatures, extremes of 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. The ICH guideline Q3B entitled 'Impurities in New Drug Products

emphasizes on providing documented evidence that analytical procedures are validated and suitable for the detection and quantitation of degradation products. It is also required that analytical methods should be validated to demonstrate that impurities unique to the new drug substance do not interfere with or are separated from specified and unspecified degradation products in the drug product. The ICH guideline Q6A, which provides note for guidance on specifications, also mentions the requirement of stability-indicating assays under Universal Tests/Criteria for both drug substances and drug products. The same is also a requirement in the guideline Q5C on Stability Testing of Biotechnological/Biological Products. Since there is no single assay or parameter that profiles the stability characteristics of such products, the onus has been put on the manufacturer to propose a stability-indicating profile that provides assurance on detection of changes in identity, purity and potency of the product. *[3,4,5]*

The following is discussion of general idea for designing stability indicating analytical method

Step 1 Physicochemical Properties of Drug

Knowledge of the physicochemical properties of the API and the formulation essential in helping to frame the development of the method. Information on various properties has been collected either through systematic program of generating the appropriate information in support of drug discovery or from a search of literature, company drug profile, spectral libraries and reports. Information about dissociation constant, partition coefficient, fluorescent properties, chromatographic behavior, spectrophotometric properties, oxidation-reduction potential are useful setting up preliminary experimental condition potential and also helpful in selecting condition of stress studies possibly in proposing degradation mechanism.*[6]*

Dissociation constant and partition coefficient can be use to develop an efficient sample extraction scheme and determine the optimum PH in mobile phase to achieve good separation. The data on fluorescence, spectrophotometric, chromatographic and oxidation reduction properties can used to determine best mean of measuring and quantifying analyte of interest. Structure of analyte, especially functional group will indicate the potential active site for degradation and susceptibility of the drug to hydrolysis, oxidation, thermal degradation etc. is determined. Compatibility study are performed to assess the stability of the when mixed with common excipient and lubricant as well as to determine any interaction between the drug and raw material. *[7]*

Step 2 Steps up Preliminary HPLC Method

Preliminary experimental condition may be adapted from official or unofficial method and from literature as a starting point. Official

method published by united states of pharmacopeia (USP) are considered validated and can be use for stability testing if it is proved stability indicating and suitable for intended purpose. New method has been created if their no suitable method available. Establishing experimental condition should be based on property of API and impurity if known. Proper column and mobile phase selection are very critical. Copious information about various HPLC column available nowadays and it is possible to select a right column for any kind of API. One of very useful source of information about column is the catalogs for vendors. Get appropriate separation condition of selecting column and mobile phase combination. Computer assisted method development can be very helpful in developing the preliminary HPLC condition quickly since the objective at this stage is quickly develop HPLC condition for subsequent method development experiment, scientist focus on separation of significant related substance instead trying to achieved good resolution all related substance. A proper experimental at the beginning will save lot of time in subsequent development stage. *[8,9]*

Step 3 Preparation of Sample Required For Method Development

SIMS is developed routinely by stressing the API under condition exceeding those normally used for accerlated stability testing. In additional demonstrating specificity in SIMS, stress testing also referred as to degradation also can be use to provide information about degradation pathway and product can could from during storage and help facilated formulation development, manufacturing and packaging. It is hard to get actual representative sample in early stage of development. Stressing API generate the sample that contain the product most likely to form under most realistic storage condition, which is in turn used to developed SIM. Generally goal of this study is to degrade the API 5-10%. Perform forced degradation study through thermolysis, hydrolysis, oxidation, and photolysis combination condition. Each force degradation sample should be analyzed by using the preliminary HPLC condition with suitable detector, most preferable PDA detector. While typical dosage form-solid (tablet/capsule), Semi solid (ointment/cream) utilize solid phase extraction (SPE) for sample preparation, especially for biosample and as a liquid liquid extraction in many U.S. Environmental protection agency method. *[10, 11]*

Step 4 Developing Separation –Stability Indicating Chromatographic Condition

The stress samples so obtained are subjected to preliminary analyses to study the number and types of degradation products formed under various conditions. For doing so, the simplest way is to start with a reversed-phase octadecyl column, preferably a new or the one in a healthy condition. Well-separated

and good quality peaks at the outset provide better confidence because of the unknown nature of products formed during stressing. It should be preferred to use water–methanol or water–acetonitrile as the mobile phase in the initial stages. The use of buffers is not suggested at this stage because as is normally required, one can extend the buffer-free mobile phase to preparative LC or LC-MS studies. Between methanol and acetonitrile, the former should be preferred due to its low cost. The wisdom from previous studies on the development of assay method for the drug can also be applied here and the organic modifier can be chosen accordingly. The solvent can be changed, if the peak shape or separation problems are seen. Initially, water: organic modifier ratio can be fixed at 50:50 or can be suitably modified so as to obtain the capacity factor of around 5–10 for the drug. As degradation products from drugs are generally polar in nature (of course with exceptions), pushing the drug peak to say 15 min or somewhat more in a 25-cm column, can result in separation of even several degradation products, when formed. The retention time can be brought earlier or pushed further by changing the mobile phase but it should not be pushed very far, as though it might lead to an overall increase in resolution (and ruggedness), but oppositely the peaks flatten out resulting in a decrease in sensitivity. Normally, the total run time should be 2.5 times more than the drug peak, at least in initial studies, and this long period is to show up any peak that would elute later to the drug peak. The detection wavelength can be set, based on the study of spectral behavior of degraded samples, as discussed earlier. The injection volume and the flow rate can be suitably adjusted based on the length of the column. Using these chromatographic conditions, one should follow the changes in all the stress samples, at various time periods. The results should be critically compared with the blank solutions injected in a similar manner. It should be observed whether the fall in drug peak is quantitatively followed by a corresponding rise in the degradation product peaks. It should not be taken as a surprise if the peak rise is not in correspondence to fall of the drug. This is because the drug and its products can have very different extinction values. Even there can be situations where no additional peak appears in the chromatogram, other than the drug. where the drug fall is clearly seen, but with no additional peak rise. Such a situation can either arise due to the formation of non-chromophoric products or due to decomposition of drug to low molecular weight fractions. In such situations, the detection at multiple wavelengths or the use of LC-MS becomes necessary. Sometimes the absence of simultaneous rise in degradation product peak might also be due to total insolubility of the product in the reaction solutions, which can be confirmed through physical observation of the reaction

mixture. In such case the product can be separated and can be injected separately using the solvent in which it is soluble to find out its retention time (RT) in the chromatogram. Later, during the final method development changes can be made in mobile phase or the sample solvent to have the product shown up in the chromatogram. Even the absence of degradation peak can happen when the product is colored and shows no UV absorption at a particular wavelength at which the analysis has been conducted. This can be verified by simple observation whether any color has developed in the reaction solution. Here also suitable adjustment in the wavelength of analysis can be made for the product to appear in the chromatogram. *[12,13]*

Step 5 Method of Optimization

The experimental condition should be optimized to get desired separation and sensitivity after getting appropriate separation. Stability indicating assay experimental condition should be achieved through the systematic examination on parameter including PH (ionic), mobile phase component and ratio, gradient, flow rate, temperature, injections volume and diluents sample type. *[9]*

Validation of Analytical Method

The validation of analytical procedures is based on the four most common types of analytical

Procedures:

- Identification tests.
- Quantitative tests for impurities' content.
- Limit tests for the control of impurities.
- Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product. *[14]*

Identification tests are intended to ensure the identity of an analyte in a sample. This is normally achieved by comparison of a property of the sample (e.g., spectrum, chromatographic behavior, chemical reactivity, etc) to that of a reference standard. Testing for impurities can be either a quantitative test or a limit test for the impurity in a sample. Either test is intended to accurately reflect the purity characteristics of the sample. Different validation characteristics are required for a quantitative test than for a limit test. Assay procedures are intended to measure the analyte present in a given sample. In the perspective of this document, the assay represents a quantitative measurement of the major component(s) in the drug substance. For the drug product, similar validation characteristics also apply when assaying for the active or other selected component(s). The same validation

characteristics may also apply to assays associated with other analytical procedures.

The various validation parameters are:

- Accuracy
- Precision (repeatability and reproducibility)
- Linearity and range
- Limit of detection (LOD)/ limit of quantitation (LOQ)
- Selectivity/ specificity
- Robustness/ ruggedness
- Stability and system suitability studies

Strategy for Validation of Methods

The validity of a specific method should be demonstrated in laboratory experiments using samples or standards that are similar to the unknown samples analyzed in the routine. The preparation and execution should follow a validation protocol, preferably written in a step by step instruction format. Possible steps for a complete method validation are listed below.

Steps in Method Validation

1. Develop a validation protocol or operating procedure for the validation
2. Define the application, purpose and scope of the method
3. Define the performance parameters and acceptance criteria
4. Define validation experiments
5. Verify relevant performance characteristics of equipment
6. Qualify materials, e.g. standards and reagents
7. Perform pre-validation experiments
8. Adjust method parameters or/and acceptance criteria if necessary
9. Perform full internal (and external) validation experiments
10. Develop SOPs (standard operating procedures) for executing the method in the routine
11. Define criteria for revalidation
12. Define type and frequency of system suitability tests and/or analytical quality control (AQC) checks for the routine
13. Document validation experiments and results in the validation.

First the scope of the method and its validation criteria should be defined. These include: compounds, matrices, type of information, qualitative or quantitative, detection and quantitation limits, linear range, precision and accuracy, type of equipment and location. The scope of the method should include the different types of equipment and the locations where the method will be run. The method's performance characteristics should be based on the intended use of the method. For example, if the method will be used for qualitative trace level analysis, there is no need to test and validate the method's linearity over the full dynamic range of the equipment. Initial parameters should be

chosen according to the analyst's best judgment. Finally, parameters should be agreed between the lab generating the data and the client using the data.*[15]* Instruments performance should be verified using generic standards, before an instrument is used to validate a method. These studies should include the approximate precision, working range and detection limits. If the preliminary validation data appear to be inappropriate, either the method itself or the equipment or the analysis technique or the acceptance limits should be changed. In this way method development and validation is an iterative process. For example, in liquid chromatography selectivity is achieved through selection of mobile phase composition. For quantitative measurements the resolution factor between two peaks should be 2.5 or higher. If this value is not achieved, the mobile phase composition needs further optimization. There are no official guidelines on the sequence of validation experiments and the optimal sequence can depend on the method itself. *[16, 17]*

Key Parameters of the Analytical Method Validation

It is important for one to understand the parameters or characteristics involved in the validation process. The various performance parameters, which are addressed in a validation exercise, are grouped as follows.

Accuracy

The accuracy of an analytical method may be defined as the closeness of the test results obtained by the method to the true value. It is the measure of the exactness of the analytical method developed. The accuracy of an analytical method may be determined by any of the following ways:

• Analyzing a sample of known concentration and comparing the measured value to the 'true' value. However, a well characterized sample (e.g., reference standard) must be used.

• Spiked – placebo (product matrix) recovery method. In this method, a known amount of pure active constituent is added to formulation blank [sample that contains all other ingredients except the active(s)], the resulting mixture is assayed, and the results obtained are compared with the expected result.

• Standard addition method. In this method, a sample is assayed, a known amount of pure active constituent is added, and the sample is again assayed. The difference between the results of the two assays is compared with the expected answer. *[18]*

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. This is usually expressed as the standard deviation or the

relative standard deviation (coefficient of variation). Precision is a measure of the degree of reproducibility or of the repeatability of the analytical method under normal operating circumstances.

Repeatability involves analysis of replicates by the analyst using the same equipment and method and conducting the precision study over short period of time while reproducibility involves precision study at different occasions, different laboratories and different batch of reagent, different analysts and different equipments. *[19]*

Determination of Repeatability

It is normally expected that at least six replicates be carried out and a table showing each individual result provided from which the mean, standard deviation and co-efficient of variation should be calculated for set of n value. The RSD values are important for showing degree of variation expected when the analytical procedure is repeated several time in a standard situation. (RSD below 1% for built drugs, RSD below 2% for assays in finished product). The ICH documents recommend that repeatability should be assessed using a minimum of nine determinations covering the specified range for the procedure (i.e. three concentrations and three replicates of each concentration or using a minimum of six determinations at 100% of the test concentration).

Determination of Reproducibility

Reproducibility means the precision of the procedure when it is carried out under different conditions-usually in different laboratories-on separate, putatively identical samples taken from

The same homogenous batch of material. Comparisons of results obtained by different analysts, by the use of different equipments, or by carrying out the analysis at different times can also provide valuable information.*[20]*

Limit of Detection

The limit of detection (LOD) of an analytical procedure is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated. It is a limit that specifies whether or not an analyte is above or below certain value. The LOD of detection of instrumental procedures is carried out by determining the signal-to noise ratio by comparing test results from the samples with known concentration of analyte with those of blank samples and establishing the minimum level at which the analyte can be reliably detected. A signal-to-noise ratio of 2:1 or 3:1 is generally accepted. The signal-to noise ratio is determined by dividing the base peak by the standard deviation of all data points below a set threshold. Limit of detection is calculated by taking the concentration of the peak of interest divided by three times the signal-to-noise ratio. For spectroscopic

techniques or other methods that rely upon a calibration curve for quantitative measurements, the IUPAC approach employs the standard deviation of the intercept (Sa) which may be related to LOD and the slope of the calibration curve, b, by LOD = 3 Sa / b. The method used to determine LOD should be documented and supported, and an appropriate number of samples should be analyzed at the time to validate the level

Quantitation Limit

Limit of quantitation (LOQ) is a parameter of quantitative assays for low levels of compounds in sample matrices such as impurities in bulk drugs and degradation products in finished pharmaceuticals. The limit of quantitation is the lowest concentration of analyte in a sample that can be determined with acceptable accuracy and precision under the stated operational conditions of the method. Like LOD, LOQ is expressed as concentration, with the precision and accuracy of the measurement also reported. Sometimes a signal-to noise ratio of 10 to 1 is used to determine LOQ. It is measured by analyzing samples containing known quantities of the analyte and determining the lowest level at which acceptable degrees of accuracy and precision are attainable. Where, the final assessment is based on an instrumental reading, the magnitude of background response by analyzing a number of blank samples and calculating the standard deviation of this response. The standard deviation multiplied by a factor (usually 10) provides an estimate of the limit of quantitation. In many cases, the limit of quantitation is approximately twice the limit of detection. *[21]*

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample.

Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage. The robustness of a method is evaluated by varying method parameters such as percent organic solvent, pH, ionic strength, temperature and determine the effect (if any) on the results of the method. The evaluation of robustness should be considered during the

development phase and depends on the type of procedure under study. If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. One consequence of the evaluation of robustness should be that a series of system suitability parameters (e.g., resolution test) is established to ensure that the validity of the analytical procedure is maintained whenever used. Examples of typical variations are stability of analytical solutions and extraction time.

Ruggedness

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different laboratories, different analysts, using operational and environmental conditions that may differ but are still within the specified parameters of the assay. The testing of ruggedness is normally suggested when the method is to be used in more than one laboratory. Ruggedness is normally expressed as the lack of the influence on the test results of operational and environmental variables of the analytical method. For the determination of ruggedness, the degree of reproducibility of test result is determined as function of the assay variable. This reproducibility may be compared to the precision of the assay under normal condition to obtain a measure of the ruggedness of the analytical method. *[22, 23]*

Conclusion

Stability indicating method is an analytical procedure that is capable discriminating between the major active pharmaceutical ingredient from any degradation product formed under define storage condition during the stability evaluation period. The use of properly designed and executed force degradation study will generate a representative sample that will in turn help to developed stability indicating HPLC method. Chromatographic factor should be evaluated to optimize the SIM-HPLC method for detection of all potentially relevant degardant. Therefore, resulting SIM-HPLC is truly fit for finding the degardant and impurity in pharmaceutical product. From the above discussed matter we concluded that the validation of developed analytical methods is critical elements in the development of pharmaceuticals. Success in these areas can be attributed to several important factors, which in turn will contribute to regulatory compliance

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