A Review On Chromatographic Method Development And Impurity Profiling

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Abstract:
Stability indicating assay method (SIAM) is a validated quantitative analytical procedure that can detect changes with time in the properties of the drug substance and drug product under defined storage conditions. It accurately measures the API without interference from other substances and is sensitive enough to detect and quantify even small amounts of degradation products/impurities. To develop a SIAM, stress testing in the form of forced degradation should be carried out as per International Conference On Harmonization (ICH) Guidelines at an early stage so that impurities and degradation products can be identified and characterized. SIAM must be discriminating and properly validated to ensure the accuracy of the long term stability testing study. Impurity profiling includes structure elucidation of organic and inorganic impurities as well as residual solvents in bulk drugs. For the characterisation of impurities various analytical techniques are used such as UV, IR, MS, NMR, Raman and some integrated spectroscopic and chromatographic techniques such as LC/MS, GC/MS, and MS/MS are also used. By using integrated techniques we can propose a structure for the impurity.

Key Words: Stability Indicating Assay Method (SIAM), degradation products, impurities, stress testing, forced degradation, ICH, impurity profiling

Introduction:

Stability Indicating Assay Methods (SIAMs) used to detect the degradants in the presence of active ingredients which are formed during storage and thus it helps in establishing of storage conditions of the drug. Determination of these impurities could be used as method for the quality control and validation of drug substances. Regulatory authorities such as US- FDA (US Food and Drug Administration) and MCA (Medicines Control Agency) insist on the impurity profiling of drugs. It is essential to know the structure of impurities in the bulk drug sample to alter the reaction condition and to reduce the quantity of impurity to an acceptable level. For newly synthesized drug substances the specification should include acceptance criteria for impurities. Stability studies can predict those impurities likely to occur in the commercial product.[(1)]

1.0 Rationale and Significance of The Study:(2-5)

Regulatory View point

Every regulatory guideline and compendia including United States Pharmacopeia (USP) says either emphatically or non-emphatically that samples of the products should be assayed for potency by use of a SIAM. Some of the leading guidelines, which discuss aboutSIAM, are listed in Table1.

Table1:Applicability of SIAM in different guidelines

Guideline	Title	Reference
ICHQ1A(R2)	Stability testing of new drug substances and products	[2]
ICHQ1B	Photostability testing of new drug substances and products	[3]
ICHQ3A(R)	Impurities in new drug substances	[4]
ICHQ3B(R2)	Impurities in new drug products	[5]

2.0 Methodologies and Techniques To Be Used:

Literature survey reveals that chromatographic methods are the best methods amongst all the methods used for analysis of stability samples.

Spectrophotometric methods are economic but non-specific[(6)], TLC methods are simple but not useful for quantitative analysis[(7)], GC methods are rapid, accurate but not suitable for thermolabile and non-volatile compounds[(8-9)]. However, HPLC methods are rapid, accurate and small quantity of mobile phase is required for analysis[(10)]. Nowadays LC-MS/ MS-MS technique is useful for quantitation of Active Pharmaceutical Ingredients (APIs) and their degradation product[(11)].

Stress Studies[12]

Stress testing is defined as stability testing of drug substances and drug products under con- ditions exceeding those that are used for ac- celerated testing. Although, all regulatory guidance documents define the concept of stress testing, they do not provide detailed in- formation about a stress testing strategy. The experimental conditions to conduct stress tests are described in a general way and not exact way. Some articles give guidelines for per- forming stress tests on a sound scientific basis among them, the approach of Singh and Bak- shiismore flexible and realistic. According to them, stress testing should be done for estab- lishment of SIAM under mild conditions, which provide maximum number of degrada- tion products. In other approaches, specific harsh conditions are followed even though they produce non- realistic degradation prod- ucts. Therefore, optimum stress conditions should be selected to avoid secondary degra- dation.

Practical Aspects of Stress Testing

The flow chart for carrying out stress testing of drug substances is given in Figure 4.

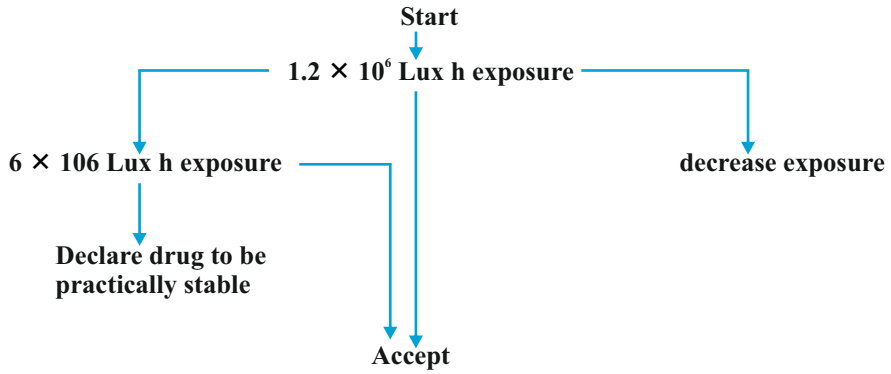

Figure: 4a Study of degradation in Photolytic condition

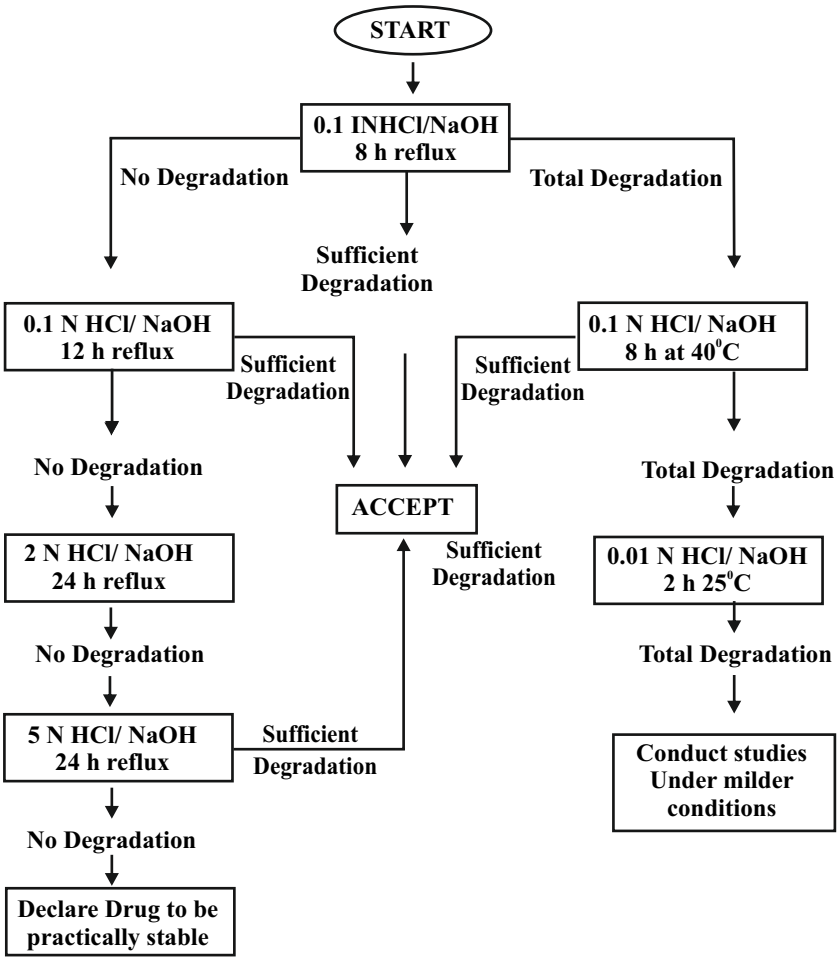

Figure: 4b Study of degradation in hydrolytic condition using acid and alkali

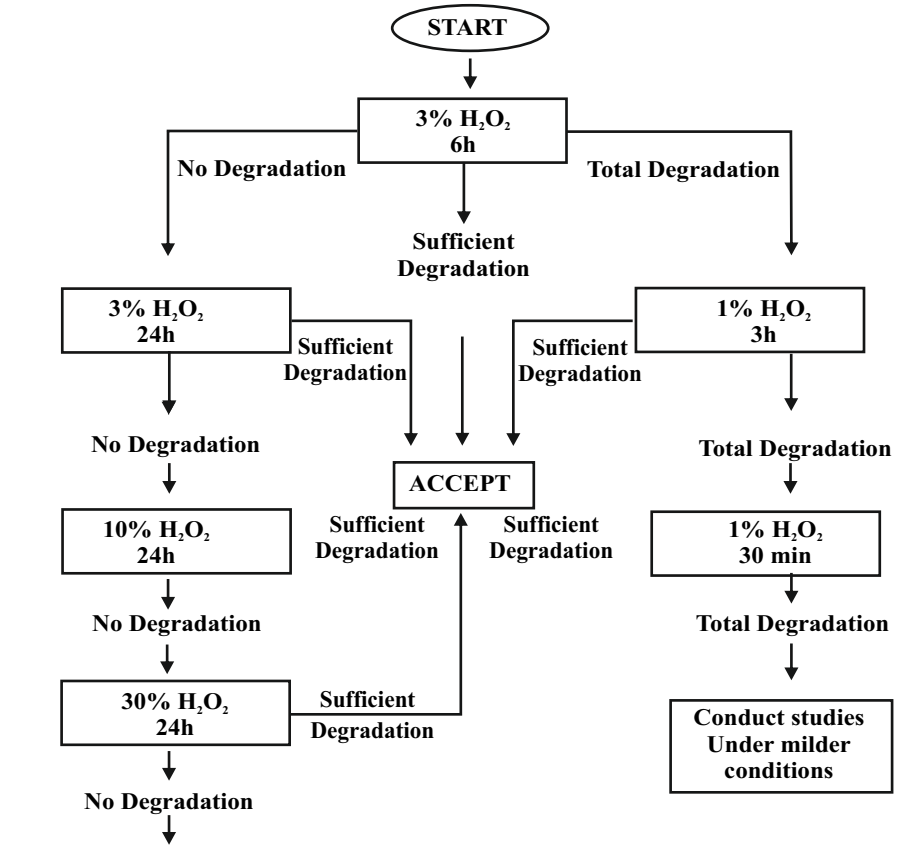

Figure: 4c Study of Degradation in Oxidative Condition using Hydrogen Peroxide.

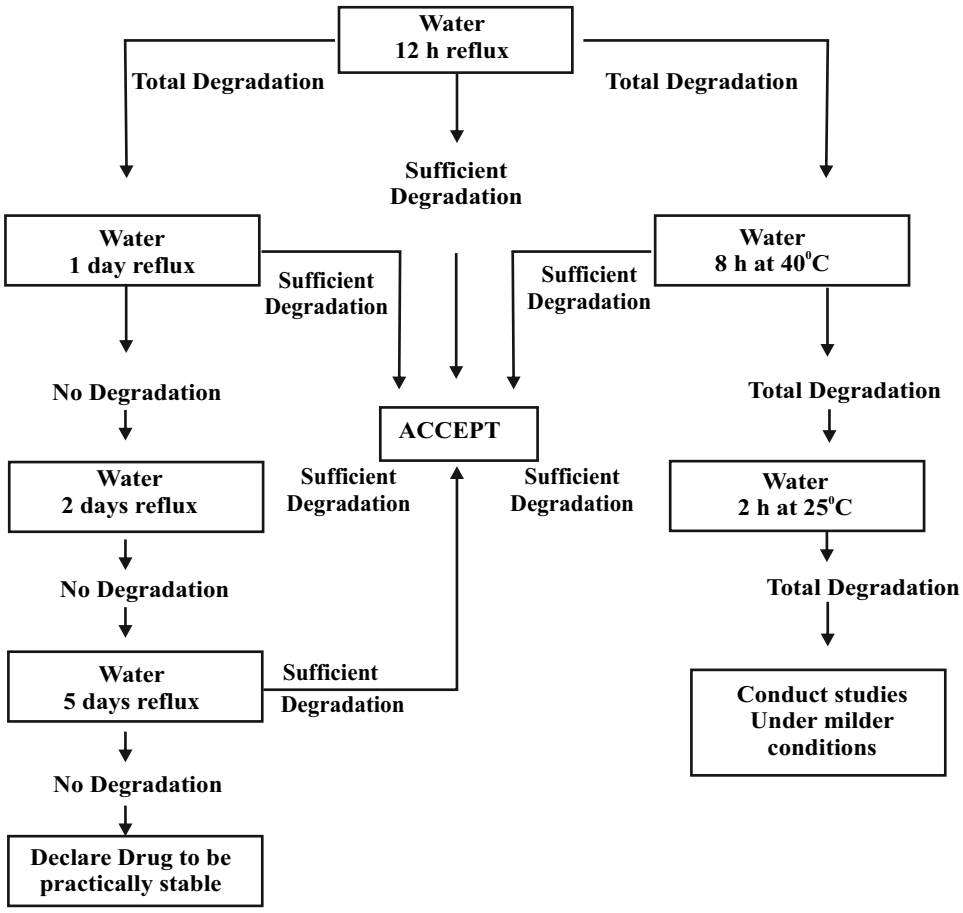

Figure: 4d Study of Degradation in hydrolytic condition using water

The studies should be conducted with a concentration greater than or equal to 1 mg/ml depending on the extent of degradation. For every stress study, it is advised to generate four samples as illustrated in Figure 5 and report the results of each. For hydrolytic studies, the reactions can simply be carried out in containers like volumetric flasks or stoppered culture tubes and stored in a water bath with thermostatic device. When studies are being carried out in multiple ampoules at one time, care should be taken on labeling. Best method is to use different color wires for ringing of ampoules.

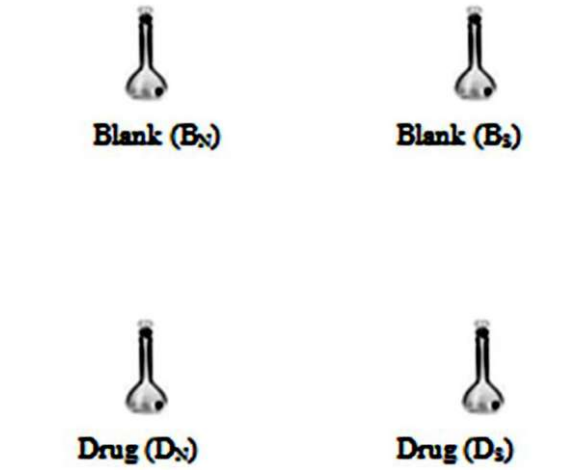

Figure: 5 Design of Study

For oxidative stress, studies should be done in a leak proof stoppered container. A caution is that the headspace left above the solution during the study should be small, for which, either the solution volume can be increased or the size of the container could be selected in such a way so that it is just sufficient to accommodate the total volume of reaction solution. For photolytic reaction, if solid drug sample is used, the study should be done in a Petri dish, where solid samples are spread evenly as a thin layer. For liquids or dissolved samples, any transparent container can be used which can give maximum exposure.

Steps involved in the development of SIAM.

For the development of validated stability indicating assay methods of drugs or their combinations following steps are used:

Step I: Critical study of the drug structure to assess the likely decomposition route.

Step II: Collection of information on physicochemical properties.

Step III: Stress (Forced decomposition)

Step IV: Preliminary separation studies on stressed samples.

Step V: Final method development and optimization.

Step VI: Characterization of degradation products.

Step VII: Validation of stability indicating assay methods.

3.0 Impurity Profiling:

As per ICH guideline Q3A impurity in a drug substance is "any component of the drug substance that is not the chemical entity defined as the drug substance "and as per ICH guideline Q3B impurity in a drug product is " any component of the drug product that is not the chemical entity defined as the drug substance or an excipient in the drug product".

As for impurity profiling, it is the common name of analytical activities with the aim of detecting, identifying or elucidating the structure of organic and inorganic impurities as well as residual solvents in bulk drugs.

Identification of impurities is done by variety of Chromatographic and Spectroscopic techniques, either alone or in combination with other techniques. The UV, IR, MS, NMR and Raman spectroscopic methods are routinely being used for characterizing impurities

The most generally used method today in the identifying or elucidating the structure of drug impurities is mass spectrometry (MS) coupled with HPLC and less generally with GC (HPLC/ MS, GC/MS). Nuclear magnetic resonance spectroscopy (NMR) is usually used ffline, but on-line HPLC/NMR, moreover HPLC/ NMR/MS, is rapidly increasing in importance.

With integrated spectroscopic and chroma- tographic information, we can propose a structure for the impurity. The next step is synthesis of the impurity with the proposed

structure. Matching chromatographic and spectral characteristics of the synthesized compound with those of the impurity furnishes final evidence for the validity of the proposed structure.

Development and validation of selective stability indicating chromatographic method can be done after successful characterisation of degradation products and the developed chromatographic method can be used for routine quality control analysis of drug samples.

Conclusion

chromatographic methods have proven to be indispensable in the development and impurity profiling of pharmaceutical compounds. The ability to separate, identify, and quantify impurities is crucial for ensuring the safety, efficacy, and quality of drug products. Various chromatographic techniques, including high-performance liquid chromatography (HPLC), gas chromatography (GC), and thin-layer chromatography (TLC), have been extensively used to detect and analyze impurities at trace levels, which is vital for regulatory compliance.

Recent advancements in chromatographic method development, such as the use of advanced stationary phases, enhanced detection techniques, and improved sample preparation strategies, have significantly increased the sensitivity, precision, and reliability of impurity analysis. These innovations have helped in the detection of both known and unknown impurities, thus ensuring the overall purity of pharmaceutical formulations.

However, challenges remain in the areas of method validation, standardization, and the need for faster, more cost-effective techniques. Continuous research is essential to address these issues and develop more efficient chromatographic approaches, particularly for complex drug formulations. Additionally, integrating chromatographic methods with other analytical techniques, such as mass spectrometry, will likely offer even more powerful solutions for impurity profiling.

Looking ahead, the continued evolution of chromatographic technologies, coupled with regulatory advancements, will play a crucial role in enhancing the safety and quality of pharmaceutical products, thereby supporting public health initiatives globally.

Source of Support: Nil

Conflict of Interest: Nil

References:

1. N. Bhavana, P.R. Likhitha, Hanumanth, P.V. Suresh, Design and Development of Stability Indicating assay methods as per ICH guidelines – A Review, International Journal of Pharma and Chemical Research, Volume 3, Issue 2, Apr-Jun 2017, pp. 252- 259
2. International Conference on Harmoniza tion, "ICH Q1A(R2):) Stability Testing of New Drug Substances and Products," Step 4 (2003).
3. International Conference on Harmonization, "ICH Q1B: Photostability Testing of New Drug Substances and Products," Step 4 (1996).
4. International Conference on Harmonization, "ICH Q3A-(R):" Impurities in New Drug Substances," Step 5 (2002).
5. International Conference on Harmonization, "ICH Q3B-® " Impurities in New Drug Products,‖ Step 5 (2003).
6. C.M. Shearer, N.J. DeAngelis, J. Pharm. Sci. 68 (1979) 1010–1012.
7. A.E.-A. El-Bayou I, A. El-Shanawany, M.E. El-Sadek, A.A. El-Sattar, J. Pharm. Biomed. Anal. 21 (1999) 867–873.
8. V.B. Patravale, V.B. Nair, S.P. Gore, J. Pharm. Biomed. Anal. 23 (2000) 623–627.
9. A.K. Hunt-Fugate, C.K. Hennessey, C.M. Kazarian,Am. J. Hosp. Pharm. 50 (1993) 1186–1187.
10. J. Lambropoulos, G.A. Spanos, N.V. Lazaridis, J.Pharm. Biomed. Anal. 19 (1999) 793–802.
11. Z. Zhao, Q. Wang, E.W. Tsai, X.-Z. Qin, D. Ip, J. Pharm. Biomed. Anal. 20 (1999) 129– 136.
12. Saranjit Singh, Monika Bakshi, Pharm. Tech. On-line 24 (2000) 1–14.