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STABILITY INDICATING ASSAY METHOD

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ABSTRACT
The main contemporary goal of stability indicating methods is to provide information about condition for stress testing so as to establish the stability of drug substances and product. HPLC is one of the most accurate method widely used for qualitative as well as qualitative analysis of drug product and is used for determining drug product stability. High performance liquid chromatography (HPLC) is an integral analytical tool in assessing drug product stability. HPLC method should be able to separate detect, & quantify the various drug-related degradants that can form on storage or manufacturing, plus detect the quality of any drug-related impurities that may be introduced during synthesis. A number of key chromatographic factors, evaluated in order to optimize the detection of all potentially relevant degradants. The present review discusses the stability indicating assay methods(SIAM) and approaches for the development of SIAM as per the current regulatory requirements.

KEYWORDS: RP-HPLC validation stability, Stability indicating method, Regulatory guidelines, degradation, Development of SIAMs, Stress testing.

INTRODUCTION

Stability indication method

Stability indication assay method is a quantitative /analytical method based on the structural and chemical properties of each active ingredient of a drug products and that will distinguish each active ingredient production so that the active ingredient content can be accurately measured. It is capable of discriminating between the intact drugs from its degradation product(S) formed under define storage conditions during the stability evaluation period. Stability indication assay should be sufficiently sensitive in order to detect and quantitateone(or) more degradation products.

The ICH stability testing guideline specifies the condition (temperature and time). the number and size of batches, testing frequencies etc. Before the introduction of the ICH guidelines, p harmaceutical companies used various conditions for long term testing often with no time or temperature control. According to FDA guideline (Guidance for industry, analytical procedures and method Validation, FDA, 2000), a stability indication method (SIM) is define as a validation analytical procedure that accurate and precisely measures active ingredients (drug substance or drug product) free from procedures for stability indicating method is to monitor results during stability indicating. It represents also a powerful tool when investigating out-of-trend (OOT) or out-of-specification (OOS) results (CDER, 2006) in quality control processes. The main objective a stability indication method to monitor results during stability studies in order to guarantee safety, efficacy and qualify.[1]

Information on the stability of the drug substance is an integral part of systematic approach to stability evaluation. The goal of stability of the program depends on the stage of development of the drug product. The formulation group also has the responsibility for recommending to the toxicology group about the stability of drug substance in the vehicle used in the animal trails. At stage the effect of pH, moisture, air, (oxygen), & light on the stability of the drug substance.[2]

Metformin is an anti-diabetic drug used to cure type 2 diabeties.[3]
1) Chemically known as N-N dimethyl –imido-dicarbon-imidicdiamide.[4]
2) It also decreases the glucose production in the lives by activating the energy regulation enzyme adenosine 5-monophosphate activated protein kinase (AMPK) which is considered as major made of metformin action.
3) It reduces the production of hepatic glucose hence improves hyperglycemia.[5]

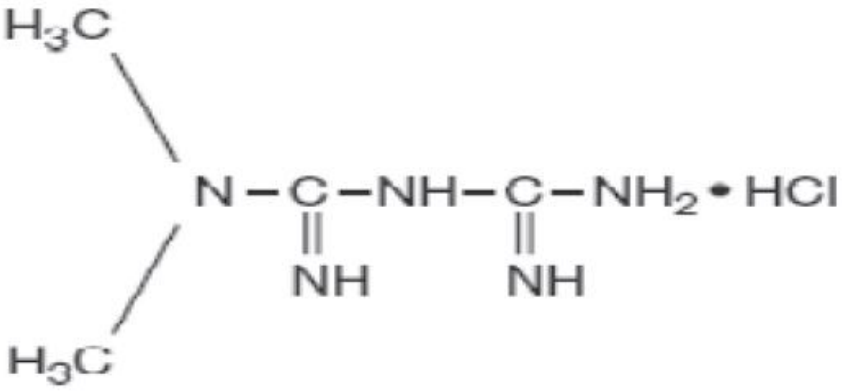


Fig.1. Structure of metformin hydrochloride

The method is expected to allow analysis of individual degradation products. The guidelines explicitly require conduct of forced decomposition studies under a variety of condition like PH, light, oxidation, dry, heart, etc., and separation drug from degradation products.

Chemically stability of pharmaceutical molecules is a matter of great concernas it affects the safety and efficacy of the drug product.[6] Stability testing of drug substance requires an accurate analytical method that quantitates active pharmaceutical ingredients (API) without interference from degradation production process impurities & with the advent of (ICH) international conference on harmonization guidelines the requirement of establishment of stability indication assay method (SIAM) has becomes more clearly mandated.[7] Knowledge of the stability of molecular helps in selecting proper formulation and package as well as providing proper storage conditions and shelf, life which essential for regulatory documentation.[6] These studies also provide information about degradation pathways and degradation products that could from during storage.[7]

Stability indicating method development Strategies'

There is no "one set fits all" formula for development stability indicating analytical method. The method development process can be visualized from a high-level process map perspective better to define the general steps encountered to achieving the end product SIM. Before beginning with actual experimentation it would be advantageous to view method development from broader some critical issues about developing stability indicating method. The following is a discussion of a genera ideal for designing stability indicating analytical method.

There are 3 critical components for a HPLC method are: sample preparation, HPLC analysis and standardization (calculation). During the preliminary method development stage all individual components should be investigated before the final method optimization. This gives the scientist a chance to component and method performance in each component and streamlines the final method optimization.[7]

Canagliflozin (Fig.2) is a oral anti- diabetic agent which belongs to a newly developed class, it has an inhibitory action on sodium –glucose co-transporter 2 (SGLT2). it received approvalby USFDA in march-2013 for treating the patient having type-II diabetis. Canagliflozia is chemically name as(2S,3R,4R,5S,6R)-2-[3-[[5-(4-fluorophenyl) thiophen-2-yl]methyl]-4methylphenyl]-6(hydroxymethyl)oxane-3,4,5-triol(4,5). SGLT2 is locatedin the proximal renal tubule and is majorly important for reabsorption of filtered glucose from its lumen, as SGLT2 is inhibited by canagliflozin the filtered glucose reabsorption decreases thereby lowering the kidney threshold for glucose and consequently increases the glucose excretion through urine.[8]

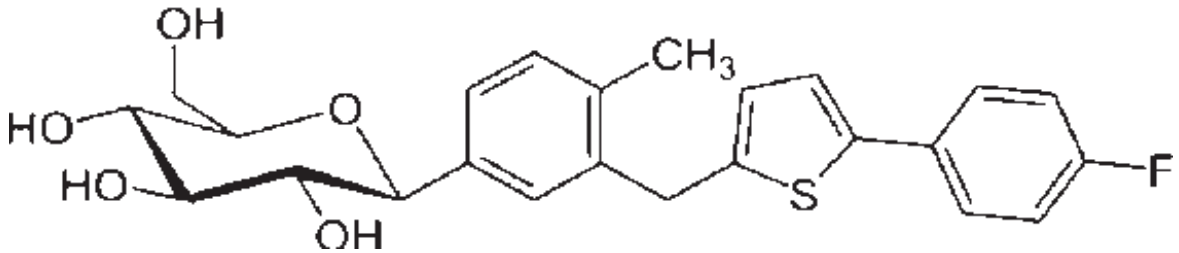


FIG- 2: Structure of Canagliflozin.

REGULATORY STATUS OF STABILITY INDICATING ASSAYS

The ICH guidelines have been incorporated as law in the US, but in reality besides these other countries are also using. The ICH guidelineQ1A 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/or 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 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 product.[9] 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 specification 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 product.[10] 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. Unfortunately, none of the ICH guidelines provides an exact definition of a stability-indicating method. Elaborate definitions of stability-indicating methodology are, however, provided in the United States-Food and Drug Administration.

(US-FDA) stability guideline of 1987 and the draftguideline of 1998.[11] Stability-indicating methods accordingto 1987 guideline were defined as the' quantitative analytical methods that are based on the characteristic structural, chemical or biological properties of each active ingredient of a drug product and that will distinguish each active ingredient from its degradaticon products so that the active ingredient content can be accurately measured. This definition in the draft guideline of 1998 reads as: validated quantitative analytical methods that can detect the changes with time in the chemical, physical, or microbiological properties of the drug substance and drug product, and that are specific so that the contents of activeingredient, degradation products, and othercomponents of interest can be accurately measured without interference.' The major changes brought in the new guideline are with Respect.

(i) Introduction of the requirement of validation, and
(ii) The requirement of analysis

A degradation products and other components, apart from the active ingredients. The requirement is also listed in World Health Organization (WHO), European Committee for Proprietary Medicinal Products and Canadian Therapeutic Products Directorate's guidelines on stability testing of well-established or existing drug substances and products.[12] Even the United States Pharmacopoeia (USP) has a requirement listed under 'Stability Studies Manufacturing' which says that samples of the products should be assayed for potency by the use of a stability-indicating assay. The requirement in such explicit manner is however absent in other pharmacopoeias.[13]

OBJECTIVE OF STABILITY STUDIES

Stability studies are performed to establish the shelf life and storage condition of API andproduct. In recently adopted stability guidelines the committee for proprietary medicinal product (CPMP) indicates the objective of stability testing is to provide evidence on how much quality of an API varies with time under influence of the variety of environmental factor such as temperature, humidity, light, oxygen. The stability of API does not mean "fix" (or) "not likely change" but it means "controlled and acceptable change". Force degradation condition stress agent concentration & time of stress are to be establishing in such a way that they effect degradation preferably 10-20% of parent constituent. Stability testing is performed for welfare of the patient and to protect reputation of producer. Requirement of regulatory agencies to provide data that may be of value formulation of other product.[14]

Define Method Objectives

There is no absolute end to the method development process. The question is what is the "acceptable method performance"? The acceptable method performance is determined by the objectives set in this step. This is one of the most important considerations often overlooked by scientists. In this section, the different end points (i.e., expectations) will be discussed.

A. Analytes: For a related substance method, determining the "significant and relevant" related substances is very critical. With limited experience with the drug product a good way to determine the significant related substances is to look at the degradation products observed during stress testing. Based on the current ICH guidelines on specifications the related substances method for active pharmaceutical ingredients (API) should focus on both the API degradation products and synthetic impurities while the same method for drug products should focus only the degradation products. In general practice unless there is any special toxicology

concerns related substances below the limit of quantitation (LOQ) should not be reported and therefore should not be investigated.

In this stage relevant related substances should be separated into 2 groups:

- **Significant related substances**: Linearity accuracy and response factors should be established for the significant related substances during the method validation. To limit the workload during method development usually 3 or less significant related substances should be selected in a method.
- **Other related substances**: These are potential degradation products that are not significant in amount. The developed HPLC conditions only need to provide good resolution for these related substances to show that they do not exist in significant levels.

B. Resolution (RS): A stability indicating method must resolve all significant degradation products from each other. Typically the minimum requirement for baseline resolution is 1.5. This limit is valid only for 2 Gaussian-shape peaks of equal size. In actual method development RS= 2.0 should be used as a minimum to account for day to day variability non-ideal peak shapes and differences in peak sizes.

C. Limit of Quantitation (LOQ): The desired method LOQ is related to the ICH reporting limits. If the corresponding ICH reporting limit is 0.1%, the method LOQ should be 0.05% or less to ensure the results are accurate up to one decimal place. However it is of little value to develop a method with an LOQ much below this level in standard practice because when the method is too sensitive method precision and accuracy are compromised.

D. Precision, Accuracy: Expectations for precision and accuracy should be determined on a case by case basis. For a typical related substance method the RSD of 6 replicates should be less than 10%. Accuracy should be within 70% to 130% of theory at the LOQ level.

E. Analysis time: A run time of about 5-10 minutes per injection is sufficient in most routine related substance analyses. Unless the method is intended to support a high-volume assay shortening the run time further is not recommended as it may compromise the method performance in other aspects (e.g., specificity, precision, accuracy).

F. Adaptability for Automation: For methods that are likely to be used in a high sample volume application it is very important for the method to be "automatable". The manual sample preparation procedure should be easy to perform. This will ensure the sample preparation can be automated in common sample preparation workstations.[7]

STEP INVOLVED DURNING THE DEVELPOMENT OF THE STABILITY INDICATING ANALYTICAL METHOD (SIAM)

A SIAM is a quantitative analytical procedure used to detect a decrease in the amount of the active pharmaceutical ingredient (API) present due to degradation. According to FDA guidelines, a SIAM is defined as a validated analytical procedure that accurately and precisely measures active ingredients (drug substance or drug product) free from potential interferences like degradation products, process impurities, excipients, or other potential impurities, and the FDA recommends that all assay procedures for stability studies be stability-indicating. During stability studies, liquid chromatography (LC) is used routinely to separate and quantitate the analyses of interest. There are three components necessary for implementing a SIAM: sample generation, method development and method validation.

A SIAMs is an estimative analytical method used to detect a trace level amount or residual levels of the API present due to degradation or designing of its synthesis route. As per the FDA regulations, a SIAMs is defined as a completely validated method that accurately and precisely measures API free from potential interferences like degradantes, biproducts, intermediates, and excipients and the FDA recommend that all assay content methodologies for stability studies be stability indicating.[15]

There are three components necessary for implementing a SIAMs.

1. Generation of degraded samples for testing selectivity of the method,
2. Method development,
3. Method validation

Step 1: Generation of degraded samples for testing selectivity of the method

Here lies one of the main concerns related to a development of a SIM, since the available guidance documents do not state the extent to which stress tests should be carried out – that is, how much stress should be applied or how much degradation should be aimed for.[16] Stress tests should generate representative samples to assess drug substance and drug product stability, provide information about possible degradation pathway and demonstrate the stability indicating power of the analytical procedures applied.

1) Determination of limit of quantification (LOQ)

In close relation to the determination of the amount of degradation is the evaluation of Limit of Detection (LOD) and Limit of Quantification (LOQ) of the method. These limits should be closely related to the reporting, identification and qualification of degradation products, as stated in ICH Q3B (R2). These thresholds are determined either as percentage of drug substance or total daily intake (TDI) of degradation product. The

analytical methods are usually expected to be validated for the ability to quantify potential degradation products and drug impurities with a LOD and LOQ at least as sensitive as the ICH threshold (see Figure. 1).

Figure 1: ICH thresholds for degradation products in New Drug Application (ICH Q3B).

Reporting threshold	
Maximum daily dose	**Threshold**
< 1.g	0.1%
>1.g	0.05%

Identification threshold	
Maximum daily dose	**Threshold**
<1mg	1.0% or 5µg TDI, whichever is lower
1mg-10mg	0.5% or 20µg TDI, whichever is lower
>10mg-2g	0.2% or 2mg TDI, whichever is lower 0.10%

Qualification threshold	
Maximum daily dose	**Threshold**
<10 mg	1.0% or 50µg TDI, whichever is lower
10mg – 100 mg	0.5% or 200µg TDI, whichever is lower
>100mg-2g	0.2% or 3mg TDI, whichever is lower
>2g	0.15%

NOTE
1. The amount of drug substance administered per day.
2. Threshold for degradation products is express either as percentage of the drug substance or as total daily intake (TDI) of the degradation product. Lower threshold can be appropriate if the degradation product unusually toxic.
3. Higher threshold should be scientifically justified.

The identification threshold (IT) varies from 0.1 to 1.0% of the labeled amount of active ingredient in the dosage form, or from 5µg to 2 mg TDI, depending on the maximum daily dosage in the product´s professional labeling. The identification threshold may be lowered for degradation products that may be exceptionally toxic. The Reporting Threshold (RT) is either 0.1% or 0.05% depending on the maxim daily dosage. For very low dose drug products, where this type of sensitivity is not attainable, even after exhaustive tentative, justification may be provided describing the failed reports. Process related drug substance impurities that are also degradation products should have the same limits as for ICH Q3B. Ideally, the same analytical methodology should be used for quality control and stability studies. The determination of out-of-specification or out-of-trend results should be more reliable, when using a SIM, since LOD and LOQ used allows detection of impurities and/or degradation products adequately. In the situation in which a new peak arises during stability study and one may expect that it should not exist and hence it would constitute a type of OOT, the use of a well-studied and well determined LOQ in a SIM, will help the applicator to decide if additional action are needed to investigate a new substance or a OOT. It should be mentioned that these thresholds are established for new drug products or New Drug Application (NDA). For Abbreviated New Drug Application (ANDA) or generic drugs, there are not specific regulations about this topic and even less, the companies dealing with these products, have background information as those obtained in the development of NDA. However, precisely because of the lack of information derived from the new drug development, the complexity and responsibility in developing/validating a SIM for an ANDA is high. Information like aqueous solubility, pH versus solubility profile, excipients compatibility studies, etc., all information that enable fully assume the knowledge of the product, will help to ensure that best (more appropriate) condition were chosen for developing a SIM, like those related to the forced degradation design.

2) Overstressing/Under stressing

Care should be taken in order to avoid over stressing or under stressing samples, with may lead to non-representative or non-purposeful degradation. So, the use of a properly designed and executed forced degradation study will generate representative samples that will help to ensure that resulting method reflects adequately long-term stability.[17] About the forced degradation (or stress test, both terms will because in the text design, it is recommended to[18] include alkaline and acidic hydrolysis, photolysis, oxidation, humidity and temperature stress. An compilation of data from literature[19] is shown at Table 1 and compiles the more often used conditions to perform forced degradation studies. These conditions can be used as a starting point in the development ofa SIM. Changing conditions to harsher or softer levels, can be applied, when too little or too much degradation are obtained. For example, in cases in which too little degradation was obtained in the hydrolyses stress, it is recommended increase concentrations to 1 Mol L-1 or higher; for oxidation stress, increase peroxide concentration to 10% or 20% (v/v) and/or time of reaction, as well as temperature. If co-solvents are necessary to increase solubility, it is

recommended the use of acetonitrile that does not work as a sensitizer in photo stability stress. Data needs to be evaluated as unusual degradants may form with co-solvents. If even not all conditions may cause degradation, Document efforts and severity of conditions and should be include in final report. By the other side, if too much degradation is detected, the severity of conditions may be decreased, by diluting acid/bases, also degradation products do not need to be described in stability studies, but SIM may assure these impurities do not interfere on degradation products determination.

Table 1: "More often" used conditions for forced degradation studies.

	Solid state	
Stress	**Condition**	**Period for time**
Heat	60%C	Up to 1 month
Humidity	75%RH	Up to 1month
Photo stability	3 mm (powder) Exposed and non-exposed samples ("control")	Follow ICH requirements (Q1B)

		Solution solid	
Stress		**Condition**	**Period of time**
Hydrolysis	Acidic	0.1 – 1 Mol L-1 HCl	Up to weeks and 60° C
	alkaline	0.1 – 1 Mol L-1 NaOH	Up to weeks and 60° C
Oxidation		H2O2 3% (v/v)	Up to 24 hours
Photostability		Exposed and non-exposed samples ("control")	Follow ICH requirements (Q1B)
Heat		60° C	Up to 1 month

3) Forced degradation studies (stress studies)

Forced degradation or stress studies are undertaken to deliberately degrade the sample. These studies are used to evaluate an analytical method's ability to measure an active ingredient and its degradation products, without interference, by generating potential degradation products. During validation of the method, drug substance are exposed to acid, base, heat, light and oxidizing agent to product approximately 10% to 30% degradation of active substance. The studies can also provide information about the degradation pathways and degradation products that could form during storage. These studies may also help in the formulation development, manufacturing, and packaging to improve a drug product. Reasons for carrying out forced degradation studies include: development and validation of stability-indicating methodology, determination of degradation pathways of drug substances and drug products, discernment of degradation products in formulations that are related to drug substances versus those that are related to non–drug substances (e.g., excipients)[20] conducted on drug substance to obtain sufficient time for identification of degradation products and structure elucidation as well as optimizing the stress conditions. An early stress study also gives timely recommendations for making improvements in the manufacturing process and proper selection of stability-indicating analytical procedures.[21]

Step 2: method development(manipulating and evaluating selectivity/specificity)

Liquid chromatography is the most appropriate technique for developing/validating a SIM. The use of diode-array-detector and additionally mass spectrometers, gives best performances for people working with SIM development. The goal is to manipulate selectivity by changing mobile phase composition, wavelength of detection and pH. Related to mobile phase pH, it can be said, that the advances in LC column technology have made possible the use of pH as a true selectivity tool for the separation of ionizable compounds.[22] Columns mechanically strong, with high efficiency and that are operate over an extended pH range, should be preferred. Acidic compounds are more retained at low pH; while basic compounds are more retained at higher pH (neutral compounds are unaffected). At traditionally used pH values (pH 4 - 8), a slight change in pH would result in a significant shift in retention. Type of chromatography used (e. g. HPLC or GC) and arrangements/detectors (GC/FID, GC/MS, LC/DAD or LC/MS) are certainly useful tools. For HPLC, different modes of chromatography can be used (normal or reversed phase, ion par or HILIC). Other powerful tool is the use of Light Scattering Detector (LSD) coupled to HPLC to monitor compounds without light absorption in UV/Vis region. Gas chromatography may only be used when no additional thermal degradation of the sample is produced (sample inlet works on high temperatures).The use of HLPC coupled to diode-array detectors (DAD) in the achievement of peak purity.[23] Usually give reasonable results, mainly related to reliable determination of the main active ingredient. It is possible to guarantee no co-elution with degradation peaks and other impurities. Indeed, the main feature of DAD detectors is that it is possible to collect spectra across a range of wavelengths at each data point collected across a peak, and through software manipulations involving multidimensional vector algebra, to compare each of the spectra to determine peak put. In this manner, DAD detectors can distinguish spectral and chromatographic differences not

readily observable by simple overlay comparisons. DAD detectors can be limited on occasion the more similar the spectra, and the lower the relative absorbance, the more difficult it can be to distinguish co-eluted compounds. MS detection overcomes many of these limitations. MS can provide unequivocal peak purity information, exact mass, structural and quantitative information depending upon the type of instrument used. MS is also a very useful tool to track peaks to selectivity manipulations in method development. As disadvantage, MS detectors cannot handle non-volatile buffers, which are frequently used as mobile phase in drug analysis. The combination of both DAD and MS on a single instrument and software platform provides the type of valuable orthogonal information required when evaluating specificity on SIM development. After determination of peak purity, in fact, the identification of degradation products and also mass balance determination usually are more complexes steps of analytical development, as in most of cases, commercial reference standards of degradation products are not available. Calculations using area-percent-normalization (area %) are not precise, since it is necessary to take into consideration the response factors (area relative to amount). Degradation products may have not the same ultra-violet spectra of that of the parent drug and even if the UV spectra are similar, the absorptivity coefficient[24] may have different values.

Case study 1

Consider the analysis of a 100 mg tablet used in medical prescriptions as 3-dose per day and coming from a long term stability study (Figure 1).

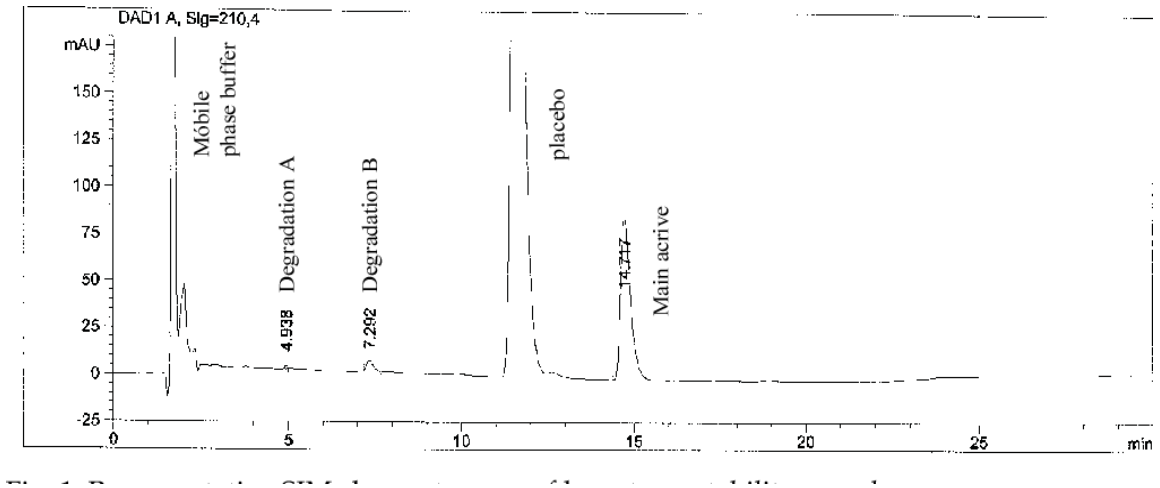


Fig: Representative SIM chromatogram of long-term stability sample

Case study 2

In this second example (representative chromatogram showed on Figure 2), one may consider that when the sample is submitted to light stress the main active degrades to seven different products and besides these seven common degradation more new two degradation can be detected in acidic stress in show (Fig: 2).[25]

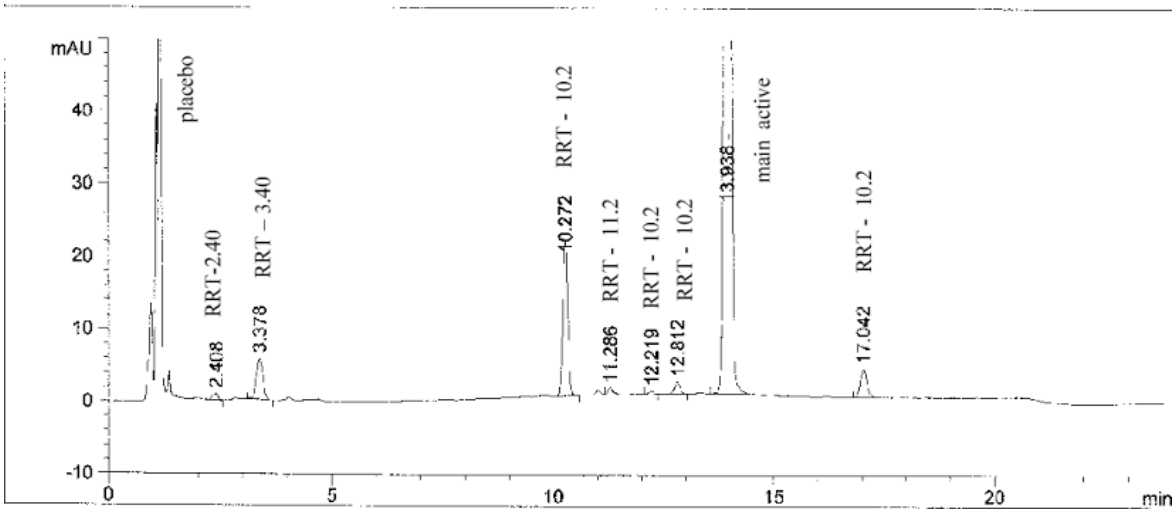


Fig. 2: SIM chromatogram of light stressed sample - 48 hours.

Mobile Phase and chromatographic conditions

Chromatographic separation was achieved on a 5 µm Inert sil ODS-2 C18 column, (150 mm x 4.6 mm) (GL Sciences, Tokyo, Japan) attached to a guard column at ambient temperature ($25\pm2_{oC}$) using a mobile phase containing a mixture of methanol as solvent A and 10mM ammonium acetate (pH-3 adjusted with glacial acetic acid) in water as solvent B in the ratio of 75:25, v/v) adjusted with a flow rate of 1.0ml/min. The mobile phase was filtered through a 0.45 µm nylon membrane and degassed with helium for 5 min.

Chromatographic methods

Because of very nature of requirement of separation of multiple components during analysis, Chromatographic methods have taken precedence over conventional methods of analysis. Other than separation of multiple components, the advantage of chromatographic methods is that these possess greater accuracy and sensitivity for even small quantities of degradation products performed. Various chromatographic methods that have been used are thin-layer chromatography (TLC), high-performance Thin-layer chromatography (HPTLC), gas chromatography (GC), HPLC (High Performance Liquid Chromatography) and newer technique like RRLC (Rapid Resolution liquid chromatography). In comparison, HPLC has been very widely employed. It has gained popularity in stability studies due to its high-resolution capacity, sensitivity and specificity. Non-volatile, thermally unstable or polar/ionic compounds can also be analyzed by this technique. Therefore, most of the SIAMs have been established using HPLC.[2]

Step-3 Method Validation

System Suitability: It is carried out to evaluate the system suitability parameters (tailing factor, resolution, theoretical plates and relative standard deviation) for replicate injections the results obtained were within the limits and are shown in (Table-1).

Specificity*:* It was performed to ensure that the response is due to single component only i.e. no co-elution exist between drug and excipients/ impurities. To perform this, a mixture of standard solution was prepared and peak purity was performed by PDA detector for metformin hydrochloride and canagliflozin. The peak purity obtained was shown in (Fig -2).

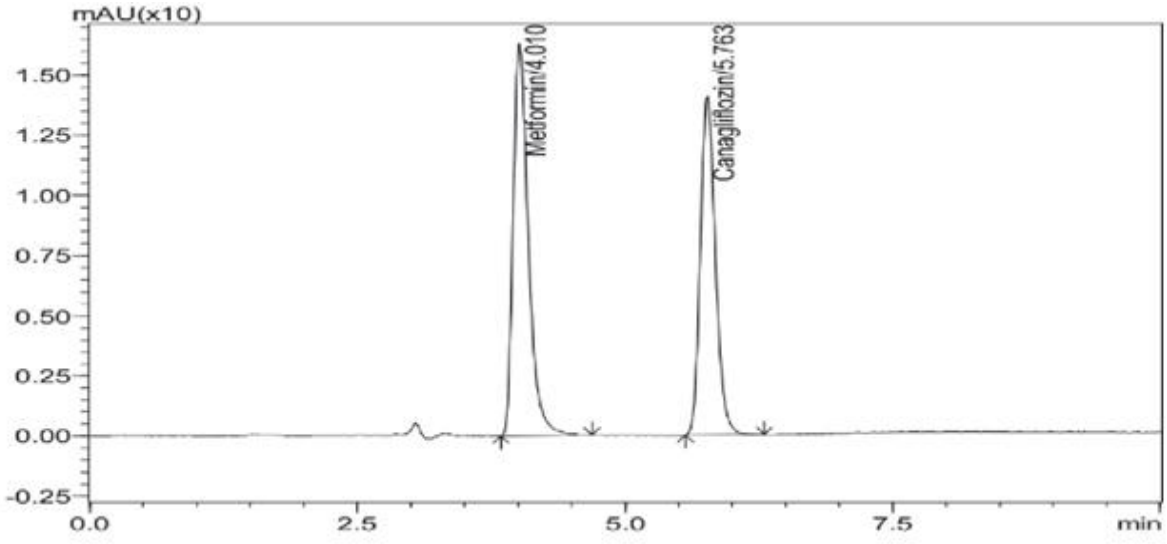


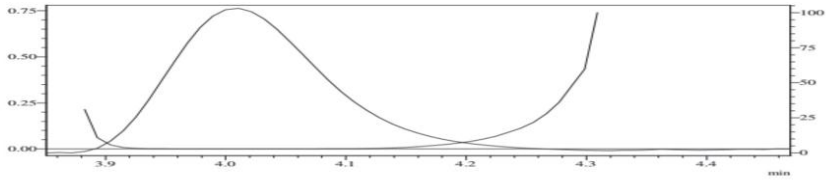
Fig. 3: Optimised chromatogram of metformin hydrochloride and canagliflozin.

- **Linearity and Range:** A series of linearity solutions for the mixture of metformin hydrochloride and canagliflozin were prepared in the concentration range of 1-80 µg/ml 20 µl of each standard was injected in triplicate and the results (chromatograms) were recorded for all the linearity standards under the optimized chromatographic conditions. The regression coefficient for both the drugs were not less than 0.999.
- **Limit of Detection (LOD) and Limit of Quantitation (LOQ):** The LOD and LOQ of the developed method were determined from the standard deviation (SD) of the response and slope (m).

Accuracy: Accuracy studies have been conducted based on the recovery of known amounts of analyte in order to determine the proposed method accuracy by standard spiking method. The recovery of the analyte was calculated by spiking a noted amount of the standard drug to the pre analyzed standard samples. Accuracy was performed at three known concentration levels of 80%, 100% and 120% of standard concentration and sample solutions were prepared in triplicate for each level.

The % recovery was found to be within the acceptance criteria of 98-102%.[26],[29]

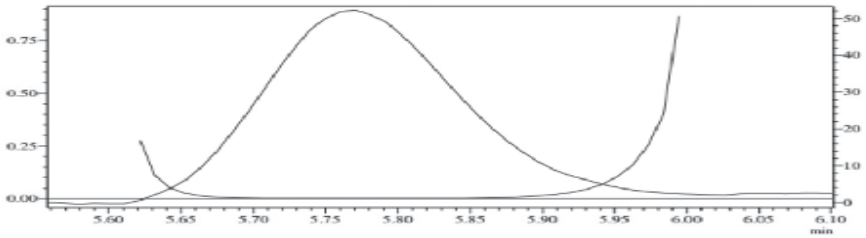
ID# : 1
Retention Time : 4.003
Compound Name : Metformin



Impurity :Not Detected
Peak purity index : 0.999993
Single point threshold : 0.999505
Minimum peak purity index : 487

Fig. 4. Peak purity curve of metformin hydrochloride

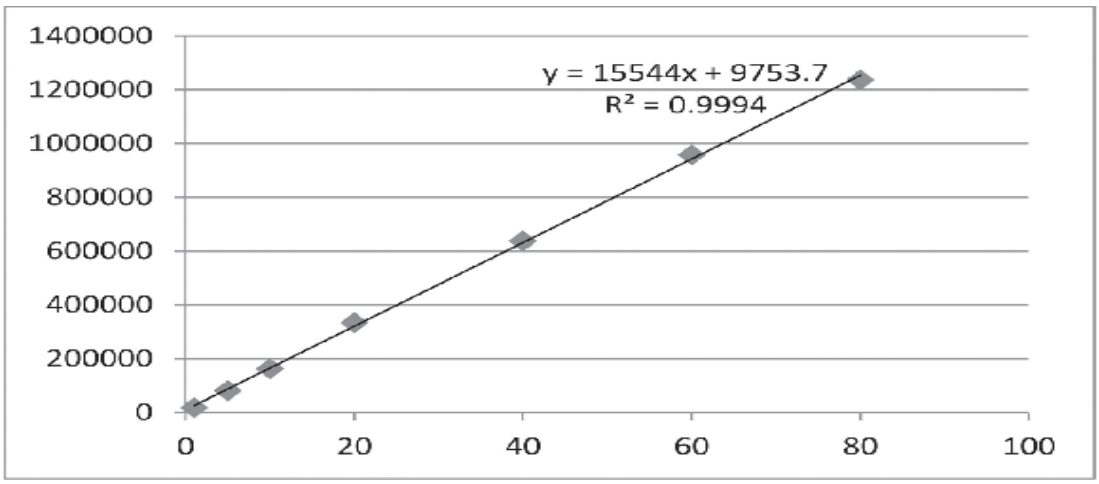
ID# : 2
Retention Time : 5.762
Compound Name : Canagliflozin



Impurity :Not Detected
Peak purity index : 0.999975
Single point threshold : 0.998787
Minimum peak purity index : 1188

Fig. 5. Peak purity curve of canagliflozin

Fig. 6: Calibration curve of metformin hydrochloride.



Validation of Analytical Methods

Validation is followed according to ICH guidelines. In validation, accuracy, precision, specificity, linearity, range, detection limit, quantitation limit, ruggedness, and robustness of the method are done. It is necessary to isolate, identify, characterize, and qualify the degradation products if they are above the identification threshold (usually 0.1%). Analytical method validation is now required by regulatory authorities for marketing authorizations and guidelines have been published. It is important to isolate analytical method validation from the selection and development of the method. Method selection is the first step in establishing an analytical method and consideration must be given to what is to be measured, and with what accuracy and precision. Method validation must have a written and approved protocol prior to use. Analytical methods used in quality control should ensure an acceptable degree of confidence that results of the analyses of raw materials, excipients, intermediates, bulk products or finished products are viable.

Experimental Design: In designing forced degradation studies, it must be remembered that more strenuous conditions than those used for accelerated studies (25°C/60% RH or 40°C/75% RH) should be used. At a minimum, the following conditions should be investigated:

- Acid and base hydrolysis,
- Hydrolysis at various pH,
- Thermal degradation,
- Photolysis, and
- Oxidation. For the drug substance and drug product, the scheme shown in **Figure 1**could be used as a guide.

The initial experiments should be focused on determining the conditions that degrade the drug by approximately 10%. The conditions generally employed for forced degradation are summarized in Fig.2.

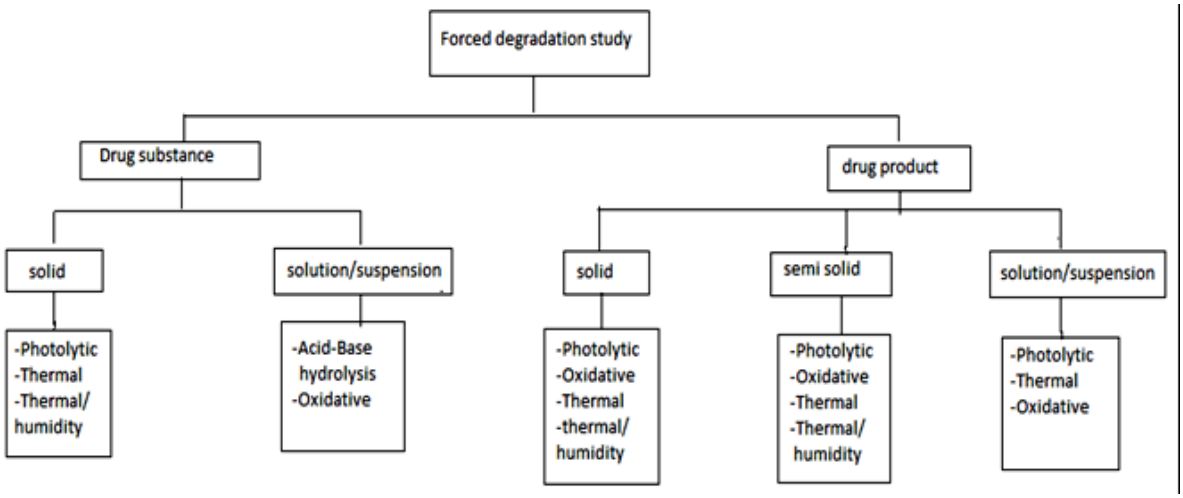


Figure 1: An illustrative diagram showing the different forced degradation condition to be used for drug substance and drug product.

	Experimental Condition	**storage condition**	**sampling time**
Degradation	Control API (no acid or base)	40 ^{0}C, 60^{0}C	1,3,5 days
	0.1N HCL	40^{0}C, 60^{0}C	1,3,5 days
	0.1N NAOH	40^{0}C, 60^{0}C	1,3,5 days
	Acid Control(no API)	40^{0}C, 60^{0}C	1,3,5 days
	Base Control(no API)	40 ^{0}C, 60^{0}C	1,3,5 days
	pH: 2,4,6,8	40 ^{0}C, 60^{0}C	1,3,5 days
Hydrolysis	3% H2O2	25 ^{0}C, 60^{0}C	1,3,5 days
	Peroxide Control	25^{0}C60^{0}C	1,3,5days
Oxidation	Azobisisobutyronitrile(AIBN)	40^{0}C,60^{0}C	1,3,5days
	AIBN Control	40^{0}C,60^{0}C	1,3,5days
photolytic	Light, 1XICH	NA	1,3,5days
	Light, 3XICH	NA	1,3,5days
	Light Control	NA	1,3,5days
	Heat chamber	60 ^{0}C	1,3,5 days
	Heat chamber	60 ^{0}C /75% RH	1,3,5 days
	Heat chamber	80 ^{0}C	1,3,5 days
	Heat chamber	80 ^{0}C /75% RH	1,3,5 days
Thermal	Heat control	Room Temp.	1,3,5 days

However, some scientists have found it practical to begin at extreme conditions (80°C or even Higher, 0.5N NaOH, 0.5N HCl, 3% H_2O_2) and testing at shorter (2, 5, 8, and 24 hrs. etc) multiple time points, thus allowing for a rough evaluation of rates of degradation. Testing at early time points may permit distinction between primary degradants and their secondary degradation products. This strategy allows for better degradation pathway determination. It must be noted that a forced degradation study is a "living process" and should be done along the developmental time line as long as changes in the stability-indicating methods, manufacturing processes, or formulation changes are ongoing.

Forced degradation is only considered complete after the manufacturing process is finalized, formulations established, and test procedures developed and qualified. The conditions listed in Table 1 are by no means exhaustive and should be adjusted by the researcher as needed to generate ~10% degradation of the API.

The nature (inherent stability/instability) of the particular drug substance will determine in which direction to adjust the stress conditions. Also, the aforementioned conditions could be used to stress the drug substance or drug product either in the solid or liquid/suspension form as applicable. For oxidative degradation with H_2O_2, at least one of the storage conditions should be at room temperature. Heating H_2O_2 solution increases the homolytic cleavage of the HO-OH bond to form the alkoxy radical. The alkoxy radical is very reactive and may come to dominate the observed degradation pathway. Adding a small quantity of methanol in a confirmatory stress experiment quenches the alkoxy radical and rules out species produced by this more aggressive oxidizing agent. Also, the formation of peroxy carboxymidic acid has been observed when acetonitrile is used as a cosolvent in H_2O_2 stress studies (in basic conditions). The peroxy carboximidic acid has activated hydroxylation reactivity, which is not representative of H_2O_2. To circumvent these problems, some research scientists always perform a parallel or alternative oxidative study using azobisisobutyronitrile (AIBN), which is a less reactive oxidant and has been shown to produce more representative degradants.[7]

Other analytic method for development of SIM

Stability-indicating methods will be characterized by potency, purity and biological activity.[26] The selection of tests is product specific. Stability indicating methods may include various methods like electrophoresisy potency, purity and biological activity(SDS-PAGE, immune electrophoresis, Westernblot, isoelectrofoccusing), High-resolution Chromatography (e.g., reversed phase chromatography, SEC, gel filtration, ion exchange, and affinity chromatography) and peptide mapping The analytical method of choice should be sensitive enough to detect impurities at low levels (i.e., 0.05% of the analyte of interest or lower) and the peak responses should fall within the range of detector's linearity. The analytical method should be capable of capturing all the impurities formed during a formal stability study at or below ICH threshold limits. Use of these techniques can provide better insight into the structure of the impurities that could add to the knowledge space of potential structural alerts for genotoxicity and the control of such impurities with tighter limits.[27] New analytical technologies that are continuously being developed can also be used when it is appropriate to develop stability indicating method. The unknown impurity, which is observed during the analysis, pharmaceutical development, stress studies and formal stability studies of the drug substances and drug product, can be separated and analyzed by using various chroma techniques like Reversed Phase High Performance Liquid Chromatography (RP-HPLC), Thin Layer Chromatography (TLC), Gas Chromatography (GC), Capillary Electrophoresis (CE), Capillary Electrophoresis Chromatography(CEC) and Super critical Fluid Chromatography (SFC). An excellent combination of hyphenated chromatographic and spectroscopic technique such as HPLC-DAD (High Performance Liquid Chromatography Photodiode Array ultraviolet Detector), LC-MS (Liquid Chromatography-Mass Spectrometry), LC-NMR (Liquid Chromatography-Nuclear Magnetic Resonance) and GCMS (Gas Chromatography-Mass Spectrometry) are used when degradants cannot be isolated in pure form. HPLC-DAD LC-MS are used to compare the RRT (relative retention time), UV spectra, mass spectra (MS/MS or MSN). Ranjit Singh et al. discussed the role of hyphenated systems for the isolation of degradants and impurities.[28],[29]

APPLICATION OF SIAMS

Stability studies are used to establish the re-test period for the active ingredient i.e., the length of time it can be stored and used without analyzing immediately before use and the shelf life of the finished product. The release and shelf life specifications for the product may differ to accommodate degradation of active ingredient or other acceptable changes, which may occur on storage. The International Conference on Harmonization (ICH) drug stability test guideline Q1A (R2) requires that analysis of stability samples should be done through the use of validated stability-indicating analytical methods (SIAMs). Additional guidance is given only for photo stability testing. It also recommends, carrying out the stress testing on drug substance to establish its inherent stability characteristics and to support the suitability of proposed analytical procedure. The validated SIAMs will be used extensively for testing the stability samples of both drug substance as well as drug product.[7]

CONCLUSION

Stress tests for developing a stability indicating method should always be designed and evaluated with common sense and chemical knowledge, keeping in mind the manufacturing process and the nature of the final drug product. The developed stability indicating assay method

is found to be easy, accurate, sensitive, specific and rapid for the simultaneous estimation of metformin hydrochloride and canagliflozin and was validated in terms of accuracy, linearity, precision, limits of detection (LOD) and Quantification (LOQ) according to ICH Q2(R1) guidelines. Stability-indicating method is an analytical procedure that is capable of discriminating between the major active (intact) pharmaceutical ingredients (API) from any degradation (decomposition) product(s) formed under defined storage conditions during] the stability evaluation period. The Automated Forced Degradation approach significantly reduces the amount of manual labor used to perform the tests and harmonizes the operational procedures of forced degradation.[4] Many combinations of test variables are possible, so it is important to use a systematic approach for developing a complex separation. First look at the variables that are both easy to change and likely to yield the most dramatic changes in selectivity. If during long-term stability studies or quality control determinations, the main active concentration decreases, mass balance investigations may start, evaluating %peak, DAD spectra and in parallel, different/contributing analytical techniques.

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