To Develop Analytical Method of Force Degradation of Desidustat on RP-HPLC

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Abstract- A simple and precise reverse phase HPLC method of force degradation was developed and validated for the analysis of desidustat in bulk drug and pharmaceutical formulation as per ICH guidelines. An analytical C18 (150X4.6mm, 5µ) eclipse SDB was used at a flow rate of 1ml/min. The mobile phase was methanol 0.1% orthophosphoric acid (72% :38% v/v). The elution was examined at 235nmand retention time for the desidustat is 5. The method can be successfully employed for the development of analytical method of force degradation of desidustat of RP-HPLC.

Keywords: desidustat, force degradation, eclipse SDB C18 (150X4.6mm, 5µ), RP-HPLC.

1. INTRODUCTION

Stability Indicating Assay Method (SIAM)

The active pharmaceutical ingredients (API) in the formulation, processing and storage may expose to variety of environmental condition like heat, humidity, light, etc. and may undergo degradation. This would lead the contamination of product with its degradation products, thus adversely affecting therapeutic efficacy and safety of drug products (DP). Therefore the stability-studies of API and its formulation is an utmost important aspect of formulation development so as to minimize its degradation and also to establish appropriate storage condition.

Regulatory authorities mainly emphasize on stability of drug in dosage form, as it exposed to various environmental conditions. The term drug stability refers to extent to which API and DP retain same properties and characteristics present at the time of manufacturing, within specific limits, throughout the period of storage. Hence it is necessary to develop a method that can establish shelf life of drug product, determine the level of certain specifications and set controlled limits for different chemical entities of drugs, and to support stability of drug used in clinical and non-clinical studies. Such method in pharmaceutical analysis is known as stability indicating assay method (SIAM)[(1)]. The knowledge gained from stress testing can be useful for-

(1) the development of stable formulation and appropriate packaging design,
(2) controlling of manufacturing and processing parameters,
(3) identification and isolation of toxic degradants during API synthesis,
(4) recommendation of appropriate storage conditions and shelf life determination, and
(5) designing and interpreting environment studies as the degradation of the drug in the environment will often be similar degradation observed during stress-testing studies.

The pharmaceutical industry is required to establish the identity and purity of all marketed drug products. Drug regulatory authorities and International Conference on Harmonization (ICH) recommended that the impurities should be isolated and characterized in drug substances and drug products, when present at the threshold levels. The identification of process related impurities and degradation products can provide insight to production of impurities and degradation mechanism.

The ICH guidelines explicitly require, conducts of forced decomposition studies under a variety of conditions like extreme pH, light, oxidation, dry heat, etc. and separation of drug from degradation products. A large number of such SIAM are reported for single ingredient drug products, however most of them fall short in meeting the current regulatory requirements.

The various ICH guidelines have been incorporated to carry the effect of regulation. The ICH guideline Q1A on Stability Testing of New Drug Substances and

Products[2] emphasizes on testing the changes which occur during storage and are likely to influence quality, safety, and/or efficacy. It is also mentioned that forced decomposition studies at temperatures in 10 ºC increments above the accelerated, extremes of pH and oxidative and photolytic conditions should be carried out on API, so as to establish the inherent stability characteristics and degradation pathways to support the suitability of the proposed analytical procedures. The ICH guidelines Q3B entitled
Impurities on New Drugs Products‟ emphasizes on documented evidence for validation of analytical procedures and suitability of the method for detection and quantitation of degradation products[3]. The ICH guidelines Q6A, which provide note for guidance on specification[4], also mention the requirement of stability-indicating assay under universal tests/criteria for both API and DP. The ICH guideline Q5C deals with Stability Testing of Biotechnological/ Biological Products[5].
Unfortunately, none of the ICH guidelines provide an exact definition of a Stability Indicating Method. Elaborate definition of Stability Indicating Methodology is provided by US-FDA stability guidelines[6] of 1987. The new US-FDA draft guidelines[7] of 1998, have recommended the major changes with respect to (i) the requirement of validation, and (ii) the analysis of degradation products and other components, apart from the active ingredients(s). The definition in the draft guideline of 1998 reads as: *"validatedquantitative analytical methods that can detect the changes with time in the chemical, physical, or microbiological properties of drug substance and drug product, and that are specific so that the contents of active ingredient, degradation products, and other components can be accurately measured without interference."*
Even the United States Pharmacopoeia (USP) has a requirement listed under stability studies in manufacturing‟, which says that samples of the products should be assayed for potency by the use of a Stability-Indicating Assay Method.[8]

Stability Testing: Regulatory Considerations
The current regulatory guidance governing forced degradation studies of pharmaceuticals are extremely general. They itemize broad principles and approaches with few practical instructions. There is no single document that comprehensively addresses issues related to stress studies such as objectives, timing, selection of stress conditions, and extent of degradation[9]. As the ICH 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[10] emphasizes that the testing of those attributes 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. 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[11]. 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[12] 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.[13]

Strategy for Stress Studies
Stability studies should include testing of those attributes of the drug substance that are susceptible to change during storage and are likely to influence quality, safety, and/ or efficacy. The testing should cover, as appropriate, the physical, chemical, biological, and microbiological attributes. Validated stability indicating analytical procedure should be applied. Whether and to what extent replication should be performed will depend on the results from validation studies.[20]

The overall strategy for developing a stability indicating method using stress testing has been expressed in Fig. 1. These studies are typically performed on both the drug substance and the formulated product.[21]

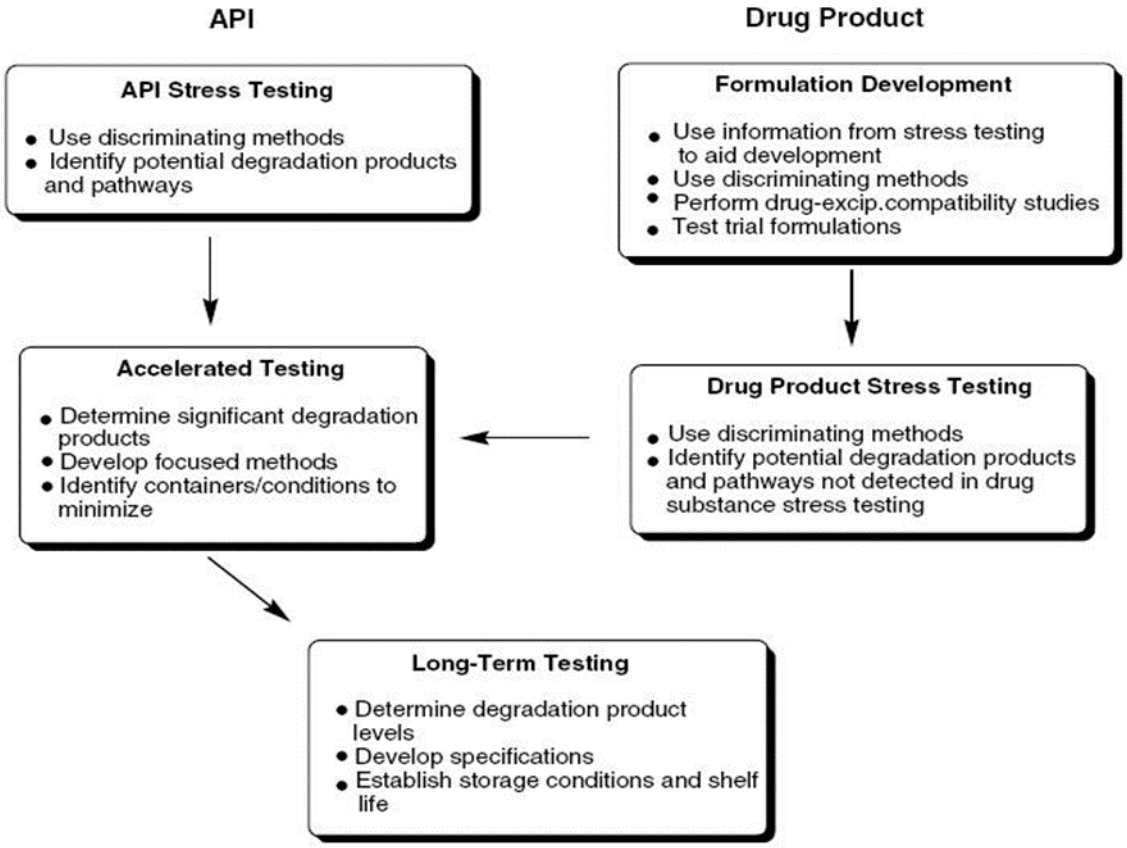


Fig. 1: Overall strategy for the prediction, identification, and control of stability related issues

Intrinsic Stability

ICH defines stress testing as an investigation of the "intrinsic stability" characteristics of the molecule. The concept of "intrinsic stability" is critically based on following aspects:

- Conditions leading to degradation (Degradation mechanisms)
- Rates of degradation (Relative or Otherwise)
- Structures of the major degradation products
- Pathways of degradation

Once these four areas have been investigated and understood, stability related issues can be identified or predicted.

Conditions Leading to Degradation (Degradation Mechanisms)

Reynolds *et al*. [22] described stress testing should include conditions that examine specifically for four main pharmaceutically relevant degradation mechanisms: (a) hydrolytic, (b) oxidative, (c) thermolytic, and (d) photolytic. The potential for these degradation pathways should be assessed in DS and DP (and/or drug - excipient mixtures). These mechanisms can be assessed in a systematic way by exposure to stress conditions of heat, humidity, photo stress (UV and VIS), oxidative conditions, and aqueous conditions across a broad pH range. [23]

Hydrolytic degradation

Drug degradation that involves reaction with water is called hydrolysis. As water is present at significant levels in many drugs (e.g., hydrates), in many excipients, and even at normal atmospheric conditions, it is not surprising that hydrolysis is a common degradation problem. Hydrolysis is affected by pH, buffer salts, ionic strength, solvent, and other additives such as complexing agents, surfactants, and excipients. [24]

Hydrolysis reactions are typically acid or base catalyzed. Acidic, neutral, and basic conditions should therefore be employed in order to induce potential hydrolytic reactions. This is especially important when the compound being tested has an ionizable functional group(s) and can exist in different ionization states

under relevant aqueousconditions. It is particularly important to be sure to test hydrolysis at unique protonation states, unless there are a large number of ionizable functional groups as is often the case with peptides and proteins. In such cases, a practical approach is to simply expose the sample to a wide pH range in defined increments (e.g., 1 pH unit).

A major challenge in designing hydrolytic stress tests is compound solubility. Many small molecule drugs are not soluble in water at the concentrations typically used for analytical evaluation (i.e., 0.1 - 1 mg/ml) across the entire pH range. Thus, either a slurry/suspension must be used to examine the hydrolytic stability of a compound or a co-solvent must be added to facilitate dissolution under the conditions of low solubility. The two most commonly used co-solvents are acetonitrile and methanol. Because methanol has the potential of participating in the degradation chemistry (e.g., acting as a nucleophile to react with electrophilic sites or intermediates in the degradation pathways), it should be used with caution (especially under acidic conditions) if the compound being tested contains a carboxylic acid, ester, or amide as these groups may react with methanol. Acetonitrile is generally regarded as an inert solvent and is typically preferable to methanol in hydrolytic stress-testing studies. However, acetonitrile is not completely inert and can participate in the degradation reactions leading to artifactual degradation results. For example, acetonitrile is known to contribute to base-catalyzed epoxidation reactions in the presence of peroxides. Acetonitrile will also degrade, in the presence of base (e.g., pH 13) and/or acid (e.g., pH 1) under elevated temperatures, to detectable levels of acetamide and/or acetic acid, which can show up as early eluting peaks (when monitoring at low wavelengths) on RP-HPLC. In the presence of radicals [e.g., generated during prolonged sonication as part of the analytical workup or in the presence of free radical initiators such as 2, 2-azobisisobutyronitrile (AIBN)], acetonitrile can be oxidized to small amounts of formyl cyanide that will readily react with nucleophiles (such as amines), resulting in a "formylation" reaction. Other co-solvents recommended for hydrolytic studies are shown in Table No. 3 [25]

Table No.3: Organic co-solvent that have been used for stress testing studies

Acidic pH	Neutral pH	Basic pH
Acetonitrile [a]	Acetonitrile [a]	Acetonitrile [a]
DMSO	NMP(N-Methylpyrrolidone)	DMSO
Acetic Acid	-	Glyme[a] (Glycol ethers)
Propionic Acid	-	Diglyme (Glycol diethers)
-	-	p-Dioxane

[a] Volatile solvent: may evaporate at higher temperatures

Co-solvents have potential to affect the degradation rates and pathways. The degradation reactions and rates involved will depend on a variety of factors such as the dielectric constant, solvent polarity, ionic strength, whether or not the solvent is protic or aprotic, the surface energy (i.e., of the solid—liquid interface in a slurry/ suspension), etc. For example, a degradation reaction involving acid-catalyzed hydrolysis with a cationic intermediate or a polarized transition state will be facilitated by a solvent with a high dielectric constant, and the addition of a co-solvent that reduces the effective dielectric constant will reduce the rate of such a reaction. Solvation of a compound in an aqueous co-solvent mixture may involve formation of a "solvent cage" of the more non-polar solvent around the compound, potentially leading to some protection from hydrolysis. Solvent composition can also affect tautomeric states of molecules, which in turn can affect both degradation rates and pathways. The effective pH of an aqueous solution will also change upon addition of a co-solvent, which can both affect the degradation rate and change the degradation pathway(s) e.g., by facilitating different protonation states.[26]

In conclusion, testing of the hydrolytic susceptibility of a DS should involve exposure to acidic, neutral, and basic conditions in the pH range of 1-13, preferably in 100% aqueous conditions. Elevated temperatures with an upper limit of 70˚C are recommended for accelerating the hydrolytic reactions. Higher temperatures can be used, but the risk of non-Arrhenius behavior increases significantly and leads to unpredictable degradation pathways. The longest recommended time period for stressing at the highest

temperature is 2 weeks, although longer times can certainly be used if desired.

Oxidative degradations

Oxidative reactions along with hydrolysis are the two most common mechanisms of drug degradation. Oxidative drug degradation pathways reactions are typically autoxidative, which is radical initiated. Radical-initiated reactions start with an initiation phase involving the formation of radicals (rate-limiting step), followed by a propagation phase and eventually a termination phase. The nature of oxidative reaction is complex. Oxidative intermediates are often thermally unstable and may decompose via alternate pathways at elevated temperatures. Increase in temperature, therefore, may not lead to predictable changes in degradation rates, and the observed oxidative rates and pathways may be different than those observed at lower temperatures. In solution, oxidative rates and pathways may be dependent on the dissolved oxygen concentration. Thus, the reaction rate in solution may actually be reduced at higher temperatures because of the decrease in oxygen content of the solvent. This may be partially overcome by bubbling oxygen or air through the solution while heating or by storing the solution under oxygen in an airtight vessel with high pressure (at least a few atmospheres). The susceptibility to oxidative degradation can be studied in solution using a radical initiator (e.g., AIBN, 40˚C, up to 1 week) and exposure to hydrogen peroxide (e.g., 0.3% hydrogen peroxide, up to 1 week at room temperature, in the dark) in separate studies. As both of these oxidative susceptibility studies are in solution, it may be useful to control the pH such that all relevant protonation states of the drug are tested. The oxidative tests could be carried out at 1 pH unit above and below of the compound being tested. Room temperature storage is sufficient for the hydrogen peroxide test. The use of higher temperatures (e.g., >30˚C) with hydrogen peroxide should be done with caution because the O-O bond is a weak bond that will readily cleave at elevated temperatures to form hydroxyl radicals, a much harsher oxidative reagent. The use of transition metals [e.g., copper (II) and iron (III) at 1-5 mM, 1-3 days] is also recommended for evaluation of oxidative susceptibility.[28]

Thermolytic degradation

Thermolytic degradation is usually thought of as degradation caused by exposure to temperatures high enough to induce bond breakage, that is, pyrolysis. Thus, any degradation mechanism that is enhanced at elevated temperatures can be considered a "thermolytic pathway".

Thermolytic pathways may lead to hydrolysis/dehydration, isomerization/epimerization, decarboxylation, rearrangements and some kinds of polymerization reactions. Hydrolytic reactions are actually a subset of thermolytic pathways. The ICH Stability guideline suggests studying the effect of temperatures in 10°C increments above the accelerated temperature test condition (i.e., 50 °C, 60 °C, etc.). It is not clear why the guideline suggests 10˚C increments, but it may be related to the importance of understanding whether or not any degradation (in the solid state) mechanism change as a result of increasing temperature. Studies with such temperature increase would be useful for constructing Arrhenius plots to allow prediction of degradation rates in the solid state at different temperatures. Waterman and Adami [29] have asserted that the relative humidity under which a solid drug product is stored is a critical variable when attempting to use the Arrhenius relationship. He showed evidence that degradation rate of formulated products (with pathways involving hydrolytic or oxidative degradation) often hold to the Arrhenius relationship if the relative humidity is held constant at the different elevated temperatures. Based on the literature and kinetic considerations, temperature up to 70 °C (at high and low humidity) should provide a rapid, reasonably predictive assessment of the solid-state degradation pathways and relative stabilities of most drug substances at lower temperatures.

Photolytic degradation

Photolytic degradation is the degradation that results from exposure to ultraviolet or visible light in the wavelength range of approximately 300-800 nm. Exposure to radiation at wavelengths <300nm is not needed because a pharmaceutical compound would not experience such exposure during its life cycle. For photolytic degradation to occur, radiation must be absorbed either by the drug substance or by the formulation. Photo degradation rates are therefore directly dependent on the amount of incident radiation

and on the amount of radiation that is absorbed by the compound or the formulation. It is important to remember that a compound may undergo photolytic degradation even if it does not itself absorb radiation in the UV or visible region. This can only happen if there is some additional agent in the formulation, intentionally or adventitiously present, that facilitates absorption.

The ICH photostability guideline (Q1 B) refers to both forced degradation studies (stress testing) and confirmatory testing. As confirmatory photostability testing is designed to be a part of the definitive, formal stability testing, it can be thought of as being analogous to an accelerated stability study. Thus, the minimum recommended exposure outlined in Q1B (i.e., 1.2 million lux-hr visible and 200W-hr/m^2 UV) is not the exposure recommended for forced degradation studies. In fact, there is no mention of recommended exposures for forced degradation studies and the design is left open. A photo exposure in the range of three to 10 times the confirmatory exposure seems a reasonable amount of photostress for forced degradation studies, remembering that photo degradation, of the compound being studied, beyond 20-30% would not be necessary or desired. It should be remembered that photo degradation products formed under stress conditions (i.e., "potential" photo degradation products) may not always be observed under confirmatory conditions. Such differences may be exacerbated by the use of different photon sources for stress testing and confirmatory studies.[30 , 31]

The stress decision trees

The decision trees are constructed for investigating different types of stress conditions for a new drug substance. The general approach taken in the construction of these flow charts is that the new drug is assumed to be labile in nature and, accordingly, it is subjected to stress conditions given for labile substances. Dependent upon the results, decision is taken on whether to increase or decrease the strength of the reaction conditions. The increase or decrease, if required, is done step-wise and those stress conditions are accepted wherever a sufficient decomposition is obtained.

Depending upon the information of degradation chemistry, the following six classes can be identified. [32]

- Extremely labile
- Very labile
- Labile
- Stable
- Very stable
- Practically stable

Table No.4: Classification system for acidic or alkaline hydrolysis

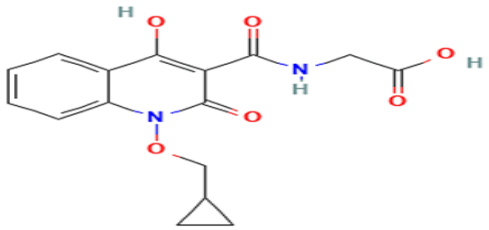
Category of Drug	Strength of acid/alkali	Time of exposure	Temperature	Extent of decomposition
Practically stable	5 N	2 days	Refluxing	None
Very stable	2 N	1 day	Refluxing	Sufficient
Stable	1 N	12 h	Refluxing	Sufficient
Labile	0.1 N	8 h	Refluxing	Sufficient
Very labile	0.01 N	8 h	40°C	Sufficient
Extremely labile	0.01 N	2 h	25°C	Sufficient

Table No.5: Classification system for hydrolysis under neutral conditions

Category of Drug	Time of exposure	Temperature	Extent of decomposition
Practically stable	5 days	Refluxing	None
Very stable	2 days	Refluxing	Sufficient
Stable	1 day	Refluxing	Sufficient
Labile	12 h	Refluxing	Sufficient
Very labile	8 h	40°C	Sufficient
Extremely labile	2 h	25°C	Sufficient

2. DRUG PROFILE OF DESIDUSTAT [2]

- Structure – Desidustat



- IUPAC Name - 2-[[1-(cyclopropyl methoxy)-4-hydroxy-2-oxoquinoline-3-carbonyl] amino] acetic acid.
- Molecular formula - $C_{16}H_{16}N_2O_6$
- Molar mass - 332.31 mol $^{-1}$
- Density - 1.5±0.1 g/cm3
- Solubility-DMF: 3mg/mL
  - DMSO: 3mg/mL
  - Ethanol: 1mg/mL
  - Methanol: 1mg/ml
- Uses - Treatment of anemia of chronic kidney disease.
- Dose: - 50 mg

2. EXPERIMENTALWORK

FORCED DEGRADATION STUDY

The force degradation study was carried out on API to ascertain the intrinsic stability of drug indifferent stress conditions, The forced degradation study was initiated using 1 mg/ml of drug indifferent stress conditions like hydrolysis at acidic, basic and neutral pH, oxidation with H_2O_2, photolysis and dry heat (thermal), The samples were withdrawn periodically like 1 h, 3h, 5h, and8 h and diluted appropriately with mobile phase to give 10 µg/ml concentration. Small amount of methanol may be used as co-solvent for solubility of drug, Stress study samples were analyzed by set HPLC method and % drug degradation was calculated using standard solution. The force degradation study was stopped at point where 5-20 % of drug was found to degrade compared to standard.

Acid Hydrolysis

A standard stock solution of Desidustat was prepared by dissolving 10 mg of Desidustat in 10 ml (1000µg/ml) of methanolic 0.1N HCl.

From above 1ml solution withdraw periodically like 1hr, 3hr, 5hr, and 8hr in 10ml volumetric flask and neutralized with 1ml of 0.1 N NaOH and remaining volume was made up by mobile phase, 1ml of the solution was taken in another 10ml volumetric flask (above solution) volume was make upto10mlbymobile phase (conc.10µg/ml) and resulting solution was used for HPLC analysis. HPLC chromatograms of forced degradation samples of desidustat are depicted in Figure no: -14

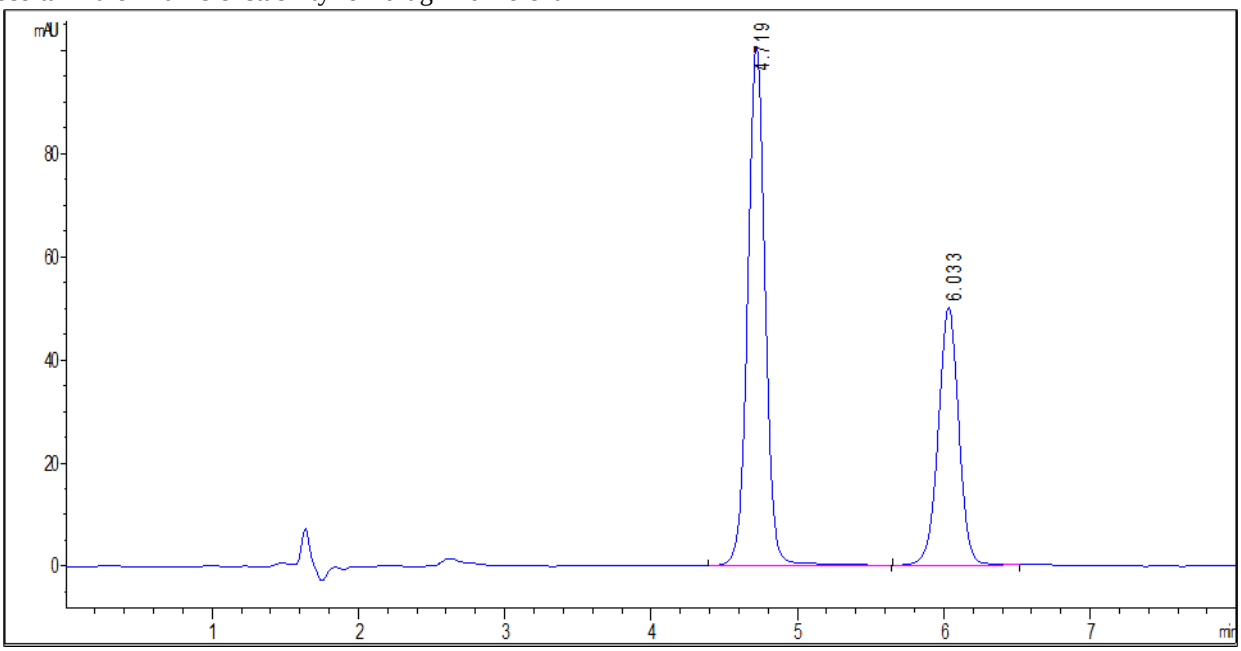


Fig14:-Chromatogram of acid hydrolytic sample (at room temp in 0.1N HCl)

Table No 15:-Observation of Acid degradation sample

Sr. No	Stress condition	Duration Hrs	Rt min	Area (std)	Area (sample)	No. of Plates N	%label claim
1	0.1N HCl (At room temp).	1Hr	4.71	1455.56	834.99	7896	57.36

In acidic hydrolysis, desidustat was found to degrade up to 57.36 % with additional peak of degradation product eluted at 6.03 min.

Alkaline Solution

A standard stock solution of Desidustat was prepared by dissolving 10mg Desidustat in 10ml (conc.1000µg/ml) of 0.1M NaOH.

From above 1ml solution withdraw periodically like 1hr, 3hr, 5hr, and 8hr in 10ml volumetric flask and neutralized with 1ml of 0.1NHCl and remaining volume was made up by mobile phase, 1ml of the solution was taken in another10ml volumetric flask (above solution) volume was make upto10ml by mobile phase (10µg/ml) and resulting solution was used for HPLC analysis. HPLC Chromatograms of forced degradation samples of desidustat are depicted in Figure No-15.

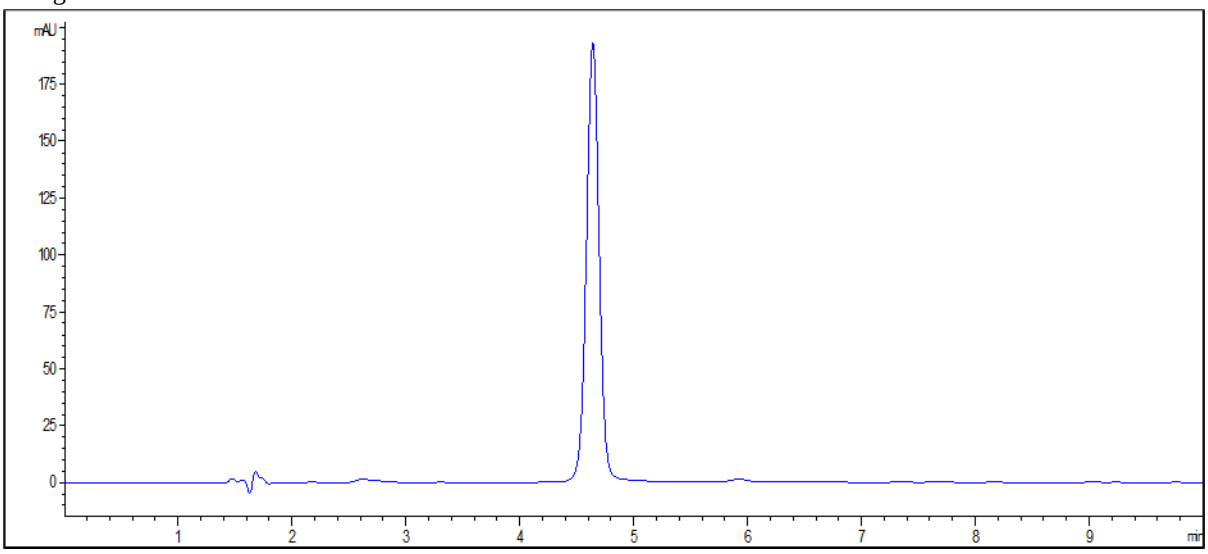


Fig 15: - Chromatogram of basic hydrolysis sample (at room temp in 0.1N NaOH for 8h)

Table No 16:-Observation of alkaline degraded sample

Sr.No	Stress condition	Duration Hrs	Temp^{O}C	RT min	Area (std)	Area (sample)	%label claim
1	0.1N NaOH	8Hrs	Room temp	4.6	1403.56	1416.27	100%

In alkaline stress condition, desidustat was found to be stable up to 8 Hr.

Oxidative Degradation

A standard stock solution of Desidustat was prepared by dissolving 10mg Desidustat in1ml 3%H_2O_2in10ml volumetric flask, remaining volume was made up to the mark with methanol (conc.1000µg/ml) and it was kept at room temperature.

From above solution 1ml was withdraw periodically 1hr, 3hr, 5hr, and 8hr diluted with mobile phase, one ml withdraws in another volumetric flask remaining volume made up with mobile phase (conc.10µg/ml) and resulting solution was used for HPLC analysis. HPLC chromatogram is depicted in figure no: -17

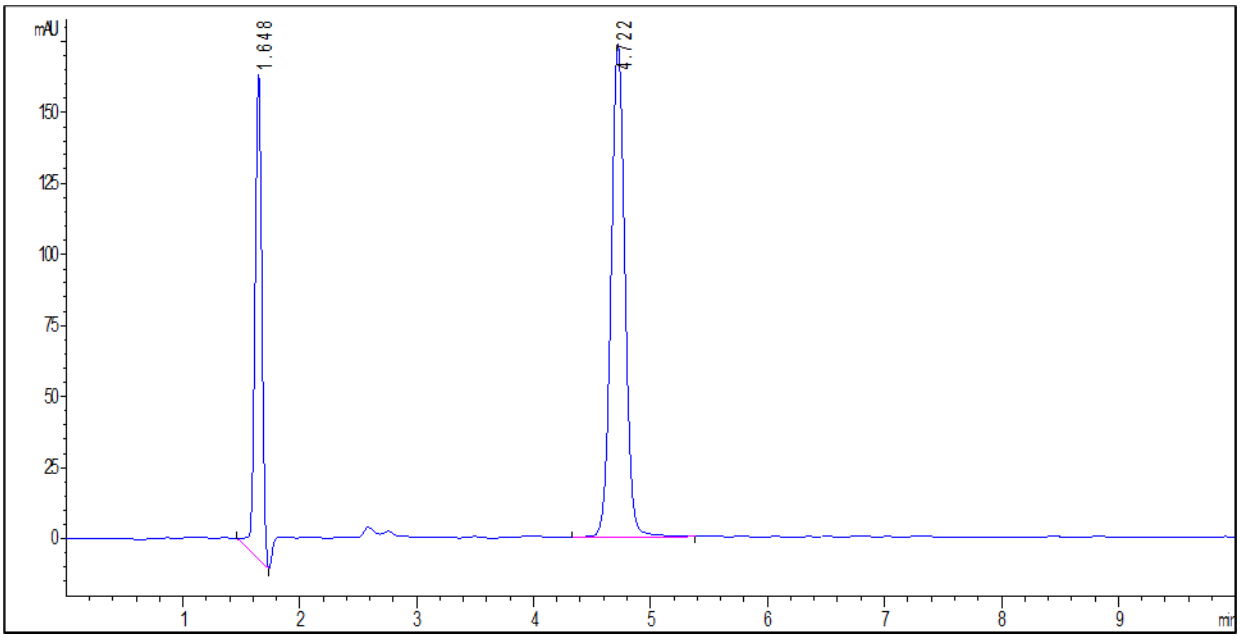


Fig 17: - Chromatogram of H_2O_2 sample (at room temp in 3%H_2O_2)

Table No 17:-Observation of Oxidative degradation sample

Sr.No	Stress condition	Duration	Temp ^{O}C	RT min	Area (std)	Area (sample)	%label claim
1	3%H_2O_2	8Hr	Room Temp	4.6	1428.91	1432.32	100%

In oxidative stress condition, desidustat was found to be stable up to 8Hr.

Thermal Degradation

A sufficient quantity of Desidustat (about 100mg) was uniformly spread a covered petri-dish andkeptinovenat100°C for different time intervals. Then, 10mg sample was withdrawn periodically on 1st, 3rd, 8th, and 15th days. Sample was transferred to 10ml volumetric flask and adjusted with methanol (1000 µg/ml). Aliquots about 1ml was withdrawn from stock and diluted to 10ml with mobile phase. From solution 1ml withdrawn and make up to 10ml with mobile phase (10 µg/ml). Final solution was used for HPLC analysis. HPLC chromatograms of forced degradation samples of desidustat are depicted in FigureNo-17

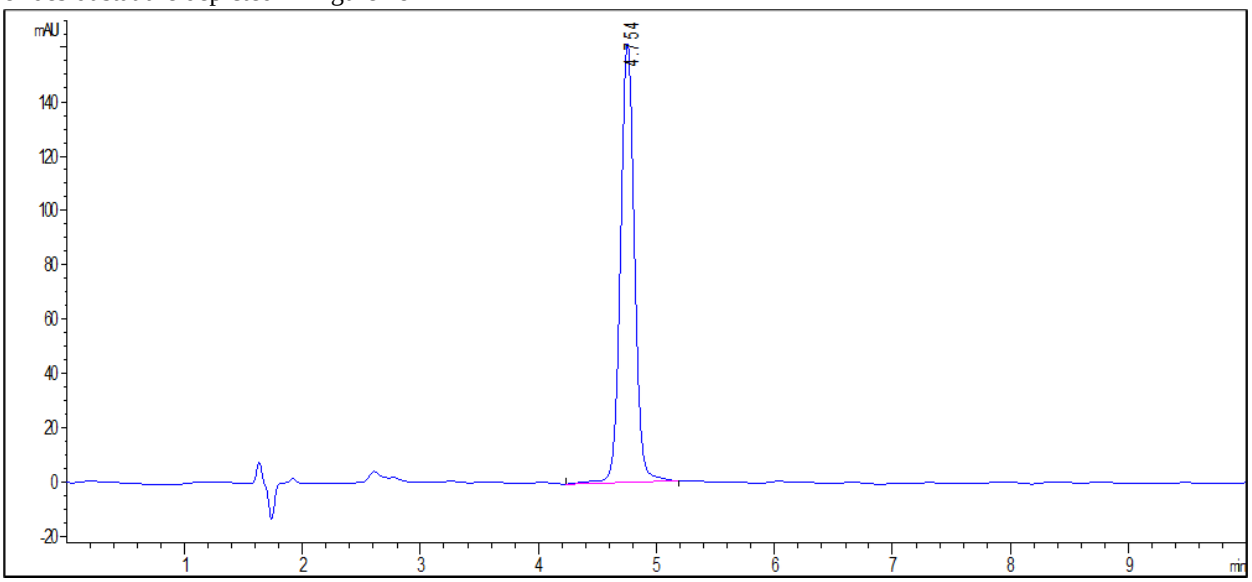


Fig17:-Chromatogram Thermal degradation (at 100^{O}C for 15th day)

TableNo18:-Observation of Thermal degradation sample

Sr. No	Stress condition	Duration day	temp	RT min	Area (std)	Area (sample)	Plate count	%label claim
1	Dry heat at 100 ^{O}C	15thday	100^{O}C	4.7	1405.1	1410.4	8148	100 %

In thermal stress condition, desidustat was found to be stable up to 15th days.

Photo Degradation

A sufficient quantity of Desidustat (about 100 mg) was uniformly spread in a covered petri -dishand kept in sunlight. Then, 10 mg sample was withdrawn periodically on 1st, 3rd, 8th and 15th day. In case photo degradation, samples were dissolved and diluted appropriately with methanol (concentration1mg/ml). Further dilution was made with mobile phase (Conc. 10 ug/ml).

Finally, the prepared stressed samples were analysed in HPLC under optimized chromatographic conditions and chromatograms were recorded. HPLC chromatograms of forced degradation samples of desidustat are depicted in Figure No-18

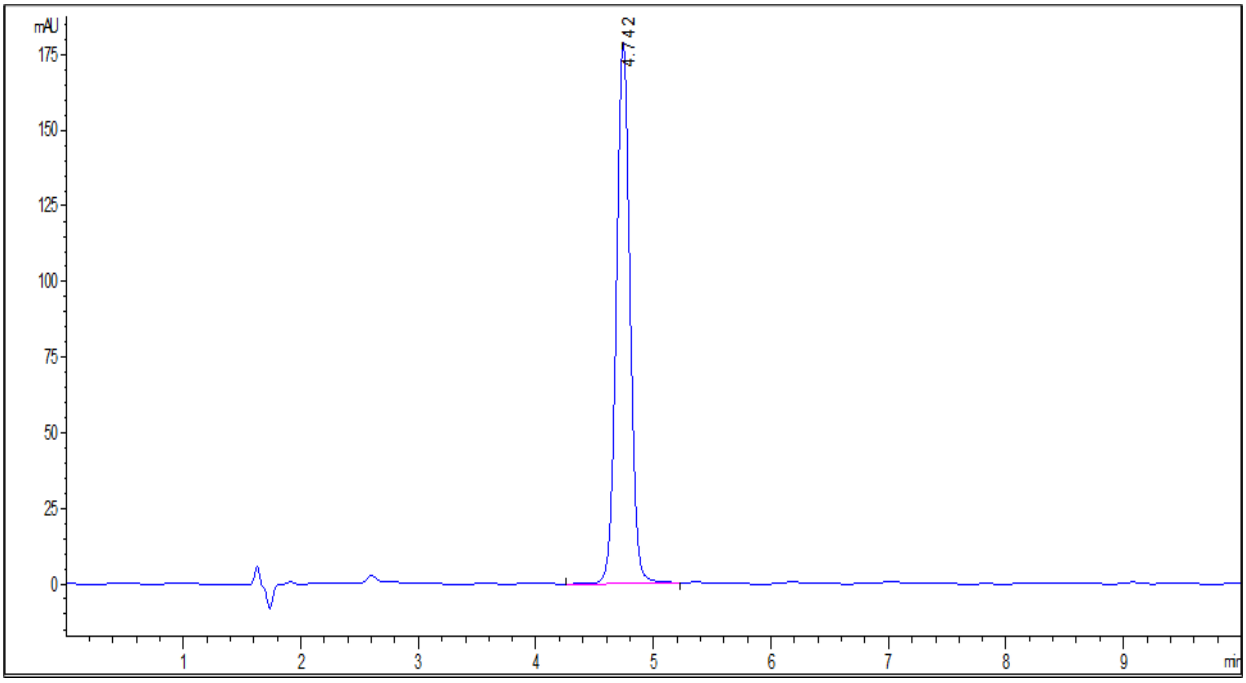


Fig18:-Chromatogram of photolysis degraded sample (in sunlight for 15th day)

Table No 19:-Observation of photodegradation sample

Sr. No	Stress condition	Duration of days	RT Min	Area of (std)	Area of sample	No. Plate	% label claim
1	Sunlight	15th day	4.7	1450.71	1452.61	8119	100%

In photolytic stress condition, desidustat was found to be stable up to 15th day.

Table no 20: - Result of forced degradation studies

Sample and condition of exposure	Retention time of degradation product (min)	% Estimation
0.1 N HCl (At room temp 1hr)	6.03	57.36%
0.1 N NaOH (At room temp 8hr)	No degradation peak	100%
3% H_2O_2 (At room temp for 8h)	No degradation peak	100%
Thermal (at 100 ^{O}C for 15th day)	No degradation peak	100%
Sunlight (15th day)	No degradation peak	100%

SUMMARY & DISCUSSION

RP-HPLC method has been developed for estimation of Desidustat from its tablet dosage form. The proposed method was validated as per ICH guidelines. Developed HPLC method was found to be suitable for regular quantitative analysis of Desidustat as per data of validation study.

Stability indicating potential of proposed method was confirmed from the data of forced degradation study. Developed HPLC method was found to be capable for simultaneous estimation of Desidustat and its degradation products. Developed method is specific and selective as peaks of degradation products were efficiently separated from standard peak and from one another. Any type of interference was not observed in chromatogram. The method was found to be robust as results did not significantly vary with deliberate modification in method parameters. Method is found to be linear and applicable over wide range of Desidustat. Method is found sensitive as per LOD and LOQ value. The method is found to be cheap as mobile phase consisted of common solvent Methanol: 0.1% ortho phosphoric acid.

As per the above discussion, it is concluded that developed RP-HPLC method can be routinely used to check stability of Desidustat for intended purpose.

Developed stability indicating HPLC method provided precious information about degradation behaviour of Desidustat at various stress conditions.

Desidustat was exposed to different stress condition like Acidic pH, Alkaline pH, H_2O_2 dry heat and light to ascertain the stability of drug.

As per results of forced degradation study, it is observed that desidustat is significantly degraded in acidic solution in 0.1N HCl at 1Hrs and one other peak was observed at Rt 6.05.

In alkaline medium, peak area of the drug peak remains unchanged over the period of time. So, it is concluded that the drug is not liable to degradation in alkaline medium.

At Oxidative stress condition, It is observed that drug is found stable to oxidative stress condition.

Stability of Desidustat was also evaluated at dry heat and sunlight. The drug was heated in oven at $100^{O}C$ and exposed to sunlight separately for 8 days. Sample was examined to assure the stability by developed HPLC method. Result of analysis revealed the stability of drug at Dry heat as no change in chromatogram was found.

As data of stability indicating HPLC method found allied and reliable, it is finally concluded that developed SIAM are suitable for the study of degradation behaviours of Desidustat for intended purpose e.g., Design and manufacture of Desidustat formulation, determination of shelf-life of formulation, selection of packaging material and storage conditions. Forced degradation study was carried out as per ICH Q1A (R2) guideline. Stress conditions were chosen as per data of HPLC stability indicating method. The selected stress conditions are shown in table: -32

Degradation Behaviours

Standard Desidustat was subjected to various stress condition to achieve the adequate degradation. Stressed sample were studied by developed RP-HPLC method to ascertain stability indicating potential of method. The chromatograms of stressed samples were evaluated for number of peak and peak purity.

TableNo: -32: - Stress Condition for HPLC of Desidustat

Stress condition	Stress solution	Exposure condition	Duration Hrs
Acid hydrolysis	0.1N HCL	Room temp ^{O}C	1Hr
Alkaline hydrolysis	0.1NaOH		8Hr
Oxidation	3% H_2O_2		8Hr
Thermal	$100^{O}C$	$100^{O}C$	8Hr
Sun light	---		8Hr

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