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FORCED DEGRADATION STUDIES ON LINAGLIPTIN TABLETS BY REVERSEPHASE HPLC METHOD

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ABSTRACT

The purpose of this research is to perform forced degradation studies of Linagliptin Tablets. A force degradation study of Linagliptin tablets 5 mg was carried out simultaneously. Force degradation is the degradation of new drug substances and drug products at condition sever than accelerated conditions. It is required to demonstrate specificity of stability indicating methods and also provides an insight into degradation pathways and degradation products of the drug substance and helps in elucidation of the structures of the degradation products. A simple and accurate method was developed for the study of the degradation pathway. The drugs substances were subjected to different degradation conditions i.e. Acid degradation, Base degradation, Oxidative degradation, photolytic degradation and Thermal degradation for different time interval. For Acid, Base and Oxidative degradation the drug substance were treated for 1st hour, 10th hour and 24 hour. For the Thermal and Oxidative degradation condition the drugs substance were treated for Day-1, Day-3 and Day-5. Force degradation studies was performed as per *ICH Guidelines, Q1A (R2), Stability testing of New Drugs Substances and products.* By Conducting the degradation study the Percentage degradation was calculated by performing assay (amount of the drug) in each condition. A simple, accurate, precise, validated and sensitive analytical RP-HPLC method was selected for analysis of drug Substances. The tablets samples were stable in the degradation study. No sample degraded more than 20%. Hence from this study it can be concluded that dosage form of Linagliptin are physically and chemically stable for their shelf life.

KEYWORDS: Forced Degradation Studies, ICH Guidelines, Linagliptin, Stress conditions.

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia, mainly due to insulin deficiency or insulin resistance. Additionally, it can rarely be caused by exposure to certain drugs, viruses, genetic mutations in the PPAR γ gene, and diseases such as cystic fibrosis and pancreatitis.[1] There were more than 537 million adults (20-79 years) are living with diabetes - 1 in 10, among which around 90% of cases is of type II Diabetes mellitus.[2] Linagliptin, 8-[(3R)-3-aminopiperidin-1-yl]-7-(but-2-yn-1yl)-3-methyl-1-[(4methylquinazolin-2- yl) methyl]-3,7-dihydro-1Hpurine-2,6-dione], is a selective, competitive dipeptidyl peptidase-4 (DPP-4) inhibitor, that was approved in 2011 by USA, Japan and Europe for the treatment of type 2 diabetes.[3] Linagliptin, as a selective and competitive dipeptidyl peptidase-4 (DPP-4) inhibitor is being used for the treatment of type 2 diabetes since 2011. Linaliptin leads to decreases in glucagon secretion and increases in insulin secretion in a glucose-dependent manner. Thus, Linagliptin provides an overall amelioration in glucose homeostasis. In addition, this promising agent inhibits DPP-4 activity with an IC50 of~1 nM and has a long duration of action (>80% DPP-4 inhibition at 24-h post dose). It is well known that Linagliptin exhibits a large safety margin, being well tolerated at doses exceeding 100-fold higher than the therapeutic dose of 5 mg.[4] The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), in collaboration with the World Health Organization (WHO), has established guidelines (ICH Q1A-E, Q3A-B, Q5C, Q6A-B) aimed at standardizing regulations across the European Union, Japan, and the United States. These guidelines facilitate the mutual acceptance of stability data that are sufficient for registration by the regulatory authorities in these jurisdictions. ICH stability studies involve testing a drug substance under storage conditions to assess its thermal stability and sensitivity to moisture. In stability testing the long-term testing should be performed over a minimum of 12 months at 25°C ± 2 °C/60% *RH* ± 5% *RH* or at 30°C ± 2°C/65% *RH* ± 5% *RH*. The intermediate and accelerated testing should span at least 6 months at 30°C ± 2°C/65% RH ± 5% RH and 40 °C ± 2 °C/75% RH ± 5% RH, respectively. However, the latter condition may be unnecessary if utilized as a long-term one. ICH stability testing for industrially fabricated medicines is both rigorous and tedious, requiring a considerable time investment to obtain preclinical stability data. For this reason, Accelerated Predictive Stability (APS) studies, carried out over a 3–4-week period and combining extreme temperatures and *RH* conditions (40–90 °C)/10–90% *RH*, have emerged as novel approaches to predict the long-term stability of pharmaceutical products in a more efficient and less time-consuming manner. The Stability studies were designed for

monitoring and evaluating the quality of Active Pharmaceutical Ingredients (API) and Finished Pharmaceutical Products (FPP) under the influence of different factors such as environmental conditions (temperature, moisture, light), API, excipients interactions, packaging materials, shelf life or container-closure systems during a certain period. The regulatory requirements are also established to evaluate degradation products of the API and impurities. Glycerol and similar excipients are susceptible to degradation with repeated use or improper storage, yet typically, any impurities produced are not routinely monitored. However, they may affect drug stability. The impact of water on the degradation of each type of solid dosage form varies significantly. In solutions and suspensions, the drug may fully or partially dissolve. However, drugs sensitive to hydrolysis are unsuitable for these liquid dosage forms. Concerning solid dosage forms, lyophilized products exhibit a greater affinity for water compared to tablets. The leftover water content in tablets after manufacturing is key, especially in moisture-sensitive drugs as it can accelerate drug degradation.[5] The guidance for stability testing of active pharmaceutical ingredients (APIs) and finished pharmaceutical products was presented in Annex 2 of the World Health Organization (WHO) Technical Report Series, No. 953, in 2009. These regulatory guidelines aim to outline the essential stability data package needed for the registration of active pharmaceutical ingredients (APIs) and finished pharmaceutical products (FPPs), superseding the earlier WHO guidelines in this domain. They reference a series of related documents published by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) (2) and other WHO guidelines.[6]

Forced degradation studies involve subjecting the new drug substance and drug product to conditions harsher than accelerated conditions. These studies reveal the chemical stability of the molecule, aiding in the development of stable formulations with appropriate storage conditions. ICH guidelines specify various degradation conditions such as light exposure, oxidation, dry heat, acidic and basic environments, and hydrolysis. Examples of forced degradation studies are illustrated in ICH Q1A, Q1B, and Q2B.[7] To demonstrate that light exposure does not lead to unacceptable changes, the intrinsic photostability characteristics of new drug substances and products should be evaluated as appropriate. Photostability testing is typically conducted on a single batch of material, chosen according to the guidelines outlined in the parent guideline's 'Selection of Batches' section Under certain circumstances, these studies should be repeated if specific variations or changes are made to the product, such as alterations in formulation or packaging. The decision to repeat these studies hinges on

the photostability characteristics established during the initial filing and the nature of the modification or alteration introduced.[8]

According to the draft guidance document given by the regulatory suggests that results of one- time forced degradation studies should be included in Phase 3 Investigational New Drugs (INDs). New Drug Application (NDA) registration requires data of forced degradation studies as forced degradation products, degradation reaction kinetics, structure, mass balance, drug peak purity, etc. The forced degradation study offers insights into the degradation pathways of the active pharmaceutical ingredient (API), both in isolation and within the drug product. It helps identify any potential formation of polymorphic or enantiomeric substances, as well as distinguishing between drug-related degradation and excipient interference.[9]

The chemical stability is crucial for maintaining the desired safety and efficacy of pharmaceutical molecules. FDA and ICH guidelines emphasize the need for stability testing data to comprehend the impact of environmental factors on the quality of drug substance and product over time. Understanding the stability of a molecule is crucial for making informed decisions regarding formulation, packaging, storage conditions, and shelf life. This knowledge is vital for regulatory documentation purposes. Forced degradation involves subjecting the novel drug substance and drug product to conditions that are more intense and severe compared to accelerated conditions, leading to their degradation. It is essential for demonstration of specificity in stability indicating methods. It not only helps establish the method's ability to accurately measure the drug's stability but also provides valuable information regarding the pathways through which degradation occurs and the resulting degradation products. Furthermore, it aids in the identification and characterization of the degradation product structures. According to the ICH guideline, stress testing aims to identify potential degradation products, assess intrinsic stability, establish degradation pathways, and validate stability indicating procedures.[10]

While the regulatory guidance offers valuable definitions and general insights into degradation studies, it tends to be broad regarding the scope, timing, and recommended practices. Although numerous guidance documents touch upon issues related to stress testing, they may not always provide specific guidance within the context of stress testing. Although available guidance documents cover topics like stereochemical stability, degradation product identification thresholds, polymorphism and crystal forms, stability of (parenteral) combination products, and mass balance, they often don't delve into these issues within

the framework of degradation studies. The FDA and International Conference on Harmonization (ICH) guidance offer limited details on strategies and principles for conducting forced degradation studies, especially regarding challenges with poorly soluble drugs and exceptionally stable compounds. Notably, the specific determination of adequate stress levels in stress testing remains unaddressed. To avoid generating degradation profiles that don't reflect real storage conditions and may not be pertinent to method development, it's crucial not to over-stress a molecule. Stress-testing conditions should thus be realistic and not excessive. In this regard, it is the amount of stress that is important and not necessarily the extent of degradation. Indeed, some compounds may not degrade significantly after considerable exposure to stress condition.[11]

Confirmatory studies, typically done in Phase III with one of the registration batches of the API for drug products, are quantitative in nature. These studies generate full mass accountability of the API, its impurities, and degradation products when carried out in late development. Furthermore, if necessary based upon the outcome of these studies, new or orthogonal methods may need to be developed to account for all observed degradation. Throughout the drug development process, forced degradation studies should be repeated as necessary, such as when there are no changes in API impurity profile, API salt, or polymorph form. Confirmatory studies are conducted when final formulation(s) and packaging are chosen. After the confirmatory studies are completed, a report on degradation products and pathways is generated and included in or used to support NDA filings.[12]

1.1 OBJECTIVE

The purposes of conducting forced degradation studies encompass the following objectives.

- Development and validation of stability-indicating methodology is undertaken.
- Determination of degradation pathways of drug substances and drug products occurs.
- The discernment of degradation products in formulations related to drug substances versus non-drug substances is conducted.
- Structure elucidation of degradation products is performed.
- The identification of impurities related to drug substances and excipients is carried out.
- The intrinsic stability of drug substances is determined.
- Reactions causing degradation of pharmaceutical products are identified.
- API stability is predicted before real-time stability data is available.
- A degradation profile is generated to mimic observations in a formal stability study under

ICHconditions.

- More stable formulations are generated.
- Understanding of the drug molecular chemistry is achieved.
- Regulatory requirements.
- Risk Assessment and Management

Overall, forced degradation studies play a critical role in ensuring the quality, safety, and efficacy of pharmaceutical products throughout their lifecycle, from development to commercialization.

1.2 Regulatory overview

Various international guidelines describe forced degradation studies. The International Committee for Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) has published a set of guidelines that have been discussed, agreed upon, and adopted by regulatory authorities in America, Europe, and Japan. In most cases, the ICH guidelines are applicable only to marketing applications for new products, excluding clinical development. However, as the conditions for forced degradation are generally defined, they can still be employed to develop stability-indicating methods during clinical development.

The same forced degradation conditions can be applied to the drug substance during development and commercialization, the ICH guidelines that are applicable to forced degradationstudies.[14]

1. ICH Q1A(R2): Stability Testing of New Drug Substances and Products
2. ICH Q1B: Photo stability Testing of New Drug Substances and Products
3. ICH Q2B: Validation of Analytical Procedures: Methodology
4. ICH Q3A: Impurities in New Drug Substances
5. ICH Q3B: Impurities in New Products
6. M4Q(R1): The common Technical Document (CTD): Quality

1.3 Stress test conditions for forced degradation studies

As per the current requirement of regulatory authorities forced degradation studies involve subjecting a pharmaceutical product and raw materials to a series of chemical and physical stresstests which are as follows:

1.4.1 Acid/Base degradation (i.e. Hydrolytic degradation)

1.4.2 Oxidative degradation

1.4.3 Photolytic degradation

1.4.4 Thermal degradation

1.4.1 Acid/Base Degradation (i.e. Hydrolytic degradation)

The degradation of a new drug in acidic and alkaline conditions through hydrolysis can be investigated by refluxing the drug in 0.1 N HCl/NaOH for 8 hours. Testing can be halted if significant degradation is observed at this stage. If there is no degradation observed in these circumstances, it is recommended to proceed by refluxing the drug in solutions with higher concentrations of acid/alkali and for an extended duration. The total degradation is seen after subjecting the drug to initial conditions, acid/alkali strength can be decreased along with decrease in the reaction temperature. In a similar manner, degradation under neutral conditions can be started by refluxing the drug in water for 12 hour. The Reflux time should be increased if no degradation is seen while studying. If the drug is found to degrade completely, both time and temperature of study can be decreased.[20]

Hydrolysis is one of the most common degradation chemical reactions over a wide range of pH. Hydrolysis procedure is a chemical process that includes decomposition of a chemical compound by reaction with water. Hydrolytic study under acidic and basic condition involves catalysis of ionizable functional groups present in the molecule. Acid and base stress testing involves forced degradation of a drug substance by exposure to acidic and basic conditions which generates primary degradants in desirable range. The selection of the type and concentrations of acid or base depends on the stability of the drug substance and drug products. Hydrochloric acid or sulfuric acids (0.1–1 M) for acid hydrolysis and sodium hydroxide or potassium hydroxide (0.1–1 M) for base hydrolysis are used as suitable reagents for hydrolysis. If the compounds for stress testing are poorly soluble in water, then co-solvents can be used to dissolve them in HCl or NaOH. The selection of co-solvent is based on the drug substance and drug products structure. Stress testing trial is normally started at room temperature and if there is no degradation, elevated temperature (50–70 °C) is applied. Stress testing should not exceed more than 7 days. The degraded sample is then neutralized using suitable acid, base or buffer, to avoidfurther decomposition.[21]

1.4.2 Oxidative degradation

Hydrogen peroxide is widely used for oxidation of drug substances in forced degradation studies but some other oxidizing agents such as metal ions, oxygen and radical initiators

(azobisisobutyronitrile, AIBN) can also be used for the study. Many drug substances undergo auto-oxidation i.e. oxidation under normal storage condition and involving ground state elemental oxygen. Therefore it is an important degradation pathway of many drugs. Auto-oxidation is a free radical reaction that requires free radical initiator to begin the chain reaction. Hydrogen peroxide, metal ions and trace level of impurities in a drug substance act as initiators for drug substance. Selection of an oxidizing agent, its concentration and condition depends on the drug substance. It is mentioned that the drug solutions are subjecting to 0.1%-3% hydrogen peroxide at neutral pH and room temperature for seven days or upto a maximum 20% degradation could potentially generate relevant degradation products. The mechanism of oxidative degradation of drug substance involves an electron transfer mechanism to form reactive anions and cations. Amines, sulphides and phenols are susceptible to electron transfer oxidation to give Noxides, hydroxylamine, sulphones and sulphoxide. The following forced degradation studies to obtain degraded samples wherever degradation possible from about 1% to 30%. For oxidation stress: treat with 1% H2O2 at less than 30°C for 30 minutes. The oxidative stress testing is initially carried out in 3% H2O2atroom temperature for 6 hour and it can be increased or decreased to achieve sufficient degradation. Co-solvent can be used to dissolve and extract the drug, where necessary.[22]

1.4.3 Photolytic degradation

Photostability testing is considered an important component of stress testing, particularly for drugs or drug products that are susceptible to light-induced degradation. It is essential to ensure that exposure to light does not cause any unacceptable changes in the drug substance or product. The ICH guidelines, specifically the ICH Q1B guideline, provide recommendations for conducting photolytic degradation testing. These guidelines suggest exposing samples to visible light under the following conditions:

- The total cumulative illumination should be at least 1.2 million lux hours.
- The integrated near ultraviolet energy should be a minimum of 200-watt hours per square meter, with a spectral distribution ranging from 320 to 400 nanometers. This range enables direct comparisons between the drug substance and drug product to be made.

Photolytic degradation, commonly induced by light, is most effectively triggered within the wavelength range of 300 to 800 nanometers. This range of light wavelengths is widely accepted as the primary source for causing photolytic degradation reactions. Moreover, study suggest that exposure of drug product to direct sunlight in their original packing container

can be done to obtain the criteria stated above.[23]

1.4.4 Thermal Stress Test/Thermal degradation

The thermal degradation study shall be carried out at more intense conditions than proposed ICH Q1A accelerated test conditions. Solid-state drug product and drug substances samples should be exposed to dry and wet heat, while liquid drug products should be exposed to dry heat. Thermal Studies may be managed at higher temperatures for a shorter period. Thermal degradation studies are conducted within the temperature range of 40°C to 80°C. The temperature of 70°C, along with low and high humidity. However, temperatures higher than 80°C may not provide accurate predictions of the degradation pathway. To evaluate the extent of degradation, the drug solution can be subjected to wet heat for several hours. It is recommended to study the impact of temperature in increments of 10°C above the standard accelerated testing range, along with humidity levels of 75% relative humidity or higher.

The role of humidity is crucial in determining potential degradants in both the finished product and active pharmaceutical ingredient. It is generally recommended to expose the samples to 90% humidity for a week to establish forced degradation samples. Testing at multiple time points can yield valuable insights into the degradation rate, as well as the primary and secondary degradation products. If the stress conditions applied result in minimal or no degradation due to the inherent stability of the drug molecule, it is important to ensure that the stress applied exceeds the energy imposed by accelerated conditions (such as 40ºC for 6 months) before concluding the stress study.[24]

Table 1.1: Some commonly used conditions used for forced degradation studies are shown in thetable below.[25]

Degradation type	Experimental conditions	Storage conditions	Sampling time (days/hour)
Acid	0.1 M HCl	40-60ºC	1,10,24 hour
	Acid Control (No. HCl)	40-60ºC	1,10,24 hour
Base	0.1 M HCl	40-60ºC	1,10,24 hour
	Base Control (No. NaOH)	40-60ºC	1,10,24 hour
Oxidative	3% H_2O_2	40-60ºC	1,10,24 hour
	No. H_2O_2	40-60ºC	1,10,24 hour
Photolytic	Direct sunlight	N/A	1,3,5
	Room	N/A	1,3,5
Thermal	Heat chamber	60-80°C/75% RH	1,3,5
	Heat control	Room temp.	1,3,5

2 Drug profile

- Common Name: Linagliptin
- Synonym: *Trajenta*.[26]
- Chemical Name: 8-[(3*R*)-3-aminopiperidin-1-yl]-7-but-2-ynyl-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]purine-2,6-dione[26]
- Molecular formula: $C_{25}H_{28}N_8O_2$[26]
- Molecular weight: 472.5 g/mol[26]
- Description: White to off white powder[26]
- Solubility: Soluble in methanol and N, N Dimethyl Formamide.[26]
- Melting point: 190-196ºC[27]

3 MATERIAL AND METHODOLOGY

3.1 Ojective

The Objective of this study is to conduct a degradation study on dosage form Linagliptin 5 mg tablets and raw material of Linagliptin. Test substances are evaluated in different stress conditions for Percentage assay.

3.2 Scope

This degradation study generates documented evidence that the tested substance has fulfilled allthe degradation criteria as per ICH guidelines.

3.3 Method of analysis of linagliptin (Raw Material and Product)

The Analytical method of Linagliptin for quantification is developed and validated as per ICH guidelines. The Validation parameters encompass various aspects, including Specificity, linearity, accuracy, precision, robustness, Intermediate precision detection limit, quantification limit, and stability studies. These all parameters are crucial in assessing the reliability and performance of analytical methods. A relative standard deviation (RSD) of less than 2% is considered acceptable, indicating good precision and reproducibility of the analytical method.[28]

Chromatography: HPLC (High Performance Liquid Chromatography)

Column: A stainless steel column of 250 mm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm).

Mobile Phase: 0.02 M Phosphate Buffer pH 4: Acetonitrile (70:30 v/v)

Flow rate: 1 ml/min. **Wavelength:** 295 nm **Injection volume:** 20 µl **Temperature:** 25ºC

Buffer Preparation: Dissolve 2.7 gm of Potassium dihydrogen orthophosphate in 1000 ml of water. Adjust the pH to 4 with phosphoric acid.

Solvent Mixture: 0.1 M HCl

3.4 Table: List of Samples its exposure days in stress conditions and its codes

S. No.	Stress conditions	Sample	Time Interval/ No. of days	Codes of sample
1.	N/A	Linagliptin Working Standard	0	Std.
2.	N/A	API	0	D0-API
3.	N/A	Linagliptin-5 mg Tablet	0	D0-L5
4.	Acidic	API	1st hour	A-D1-API
5.			10th hour	A-D10-API
6.			24th hour	A-D24- API
7.		Linagliptin-5 mg Tablet	1st hour	A-D1-L5
8.			10th hour	A-D10-L5
9.			24th hour	A-D24-L5
10.	Basic	API	1st hour	B-D1-API
11.			10th hour	B-D10-API
12.			24th hour	B-D24- API
13.		Linagliptin-5 mg Tablet	1st hour	B-D10-L5
14.			10th hour	B-D10-L5
15.			24th hour	B-D24-L5
16.	Oxidative	API	1st hour	O-D1-API
17.			10th hour	O-D10-API
18.			24th hour	O-D24- API
19.		Linagliptin-5 mg Tablet	1st hour	O-D1-L5
20.			10th hour	O-D10-L5
21.			24th hour	O-D24-L5
22.	Thermal	API	Day-1	T-D1-API
23.			Day-3	T-D3-API
24.			Day-5	T-D5- API
25.		Linagliptin-5 mg Tablet	Day-1	T-D1-L5
26.			Day-3	T-D3-L5
27.			Day-5	T-D5-L5
28.	Photolytic	API	Day-1	P-D1-API
29.			Day-3	P-D3-API
30.			Day-5	P-D5- API
31.		Linagliptin-5 mg Tablet	Day-1	P-D1-L5
32.			Day-3	P-D3-L5
33.			Day-5	P-D5-L5

$$\frac{\text{Area of Test}}{\text{Area of Std.}} \times \frac{\text{Wt. of Std.}}{100} \times \frac{1}{100} \times \frac{100}{\text{Weight of Test}} \times \frac{25}{1} \times \frac{\text{Potency of Std.}}{100} \times \frac{100 - \text{WC of WS}}{100}$$ X Avg. wt. of

3.5 Calculation of assay

Percentage assay =

Where,

Std. = Working StandardWC= Water content

Wt. = Weight Avg. = Average

4 RESULT

As per above dilutions and formula percentage assay of each stage and analysis.

4.1 Analysis on day 0

Table 4.1: Calculation's and data of Analysis of Samples on Day 0.

Standard weight (mg)	Average area of standard	Sample	Weight of test(mg)	Area oftest	% Assay	Average % assay
40.22	166631	API (D0-API)	40.92	173299	100.61	100.85
			40.34	171661	101.09	
		Linagliptin-5 mg Tablet (D0-L5)	301.85	169251	102.74	103.37

4.2 Analysis on day 1 and 1st hour

Std. weight (mg)	Average area of standard	Stress condition	Time interval	Sample	Weightof test (mg)	Area of test	% Assay	Average % assay
40.22	166631	Acidic	1st hour	API (A-D1-API)	40.92	173835	100.92	100.73
					40.34	170747	100.55	
				Linagliptin-5 mg Tablet(A-D1-L5)	301.85	167518	101.69	102.36
					306.88	172553	103.03	
40.22	166631	Basic	1st hour	API (B-D1-API)	40.92	173025	100.45	100.68
					40.34	171374	100.92	
				Linagliptin-5 mg Tablet(A-B1-L5)	301.85	168773	102.45	102.48
					306.88	171706	102.52	
40.22	166631	Oxidative	1st hour	API (O-D1-API)	40.92	171273	99.43	99.97
					40.34	170670	100.51	
				Linagliptin-5 mg Tablet (O-D1-L5)	301.85	168547	102.31	102.65
					306.88	172497	102.99	
40.15	172956	Thermal	Day-1	API (T-D1-	40.97	178162	99.35	99.44

				API)	40.73	177441	99.53	
				Linagliptin-5 mg Tablet(T-D1-L5)	309.26	172658	98.69	98.92
					308.53	174178	99.15	
40.70	172816	Photolytic	Day-1	API (A-D1-API)	40.67	176099	100.36	99.88
					40.19	172346	99.40	
				Linagliptin-5 mg Tablet (P-D1-L5)	313.20	172896	98.69	98.92

4.3 Analysis on day 3 and 10th hour.

Std. weight (mg)	Average area of standard	Stress condition	Time interval	Sample	Weightof test (mg)	Area of test	% Assay	Average % assay
40.07	166007	Acidic	10th hour	API (A-D10-API)	40.92	173531	100.74	100.10
					40.34	168876	99.45	
				Linagliptin-5 mg Tablet(A-D10-L5)	301.85	167789	101.85	101.48
					306.88	169348	101.11	
40.07	166007	Basic	10th hour	API (B-D10-API)	40.92	172334	100.05	100.69
					40.34	172062	101.33	
				Linagliptin-5 mg Tablet(A-B10-L5)	301.85	166908	101.32	101.84
					306.88	171438	102.36	
40.07	166007	Oxidative	10th hour	API (O-D10-API)	40.92	172129	99.93	99.27
					40.34	167459	98.62	
				Linagliptin-5 mg Tablet (O-D10-L5)	301.85	166854	101.28	100.83
					306.88	168102	100.37	
40.14	171188	Thermal	Day-3	API (T-D3-API)	41.32	176013	98.30	98.11
					40.95	173745	97.91	
				Linagliptin-5 mg Tablet(T-D3-L5)	310.26	169552	97.27	97.10
					311.65	169716	96.93	
40.26	172341	Photolytic	Day-3	API (A-D3-API)	40.97	175263	98.35	98.54
					40.85	175411	98.73	
				Linagliptin-5 mg Tablet (P-D3-L5)	310.26	171545	98.05	98.33
					311.65	173297	98.61	

4.4 Analysis on day 5 and 24th hour

Std. weight (mg)	Average area of standard	Stress condition	Time interval	Sample	Weightof test(mg)	Area of test	% Assay	Average % assay
40.09	165153	Acidic	24th hour	API (A-D24-API)	40.92	168986	98.66	98.53
					40.34	166148	98.40	
				Linagliptin-	301.85	161302	98.47	98.30

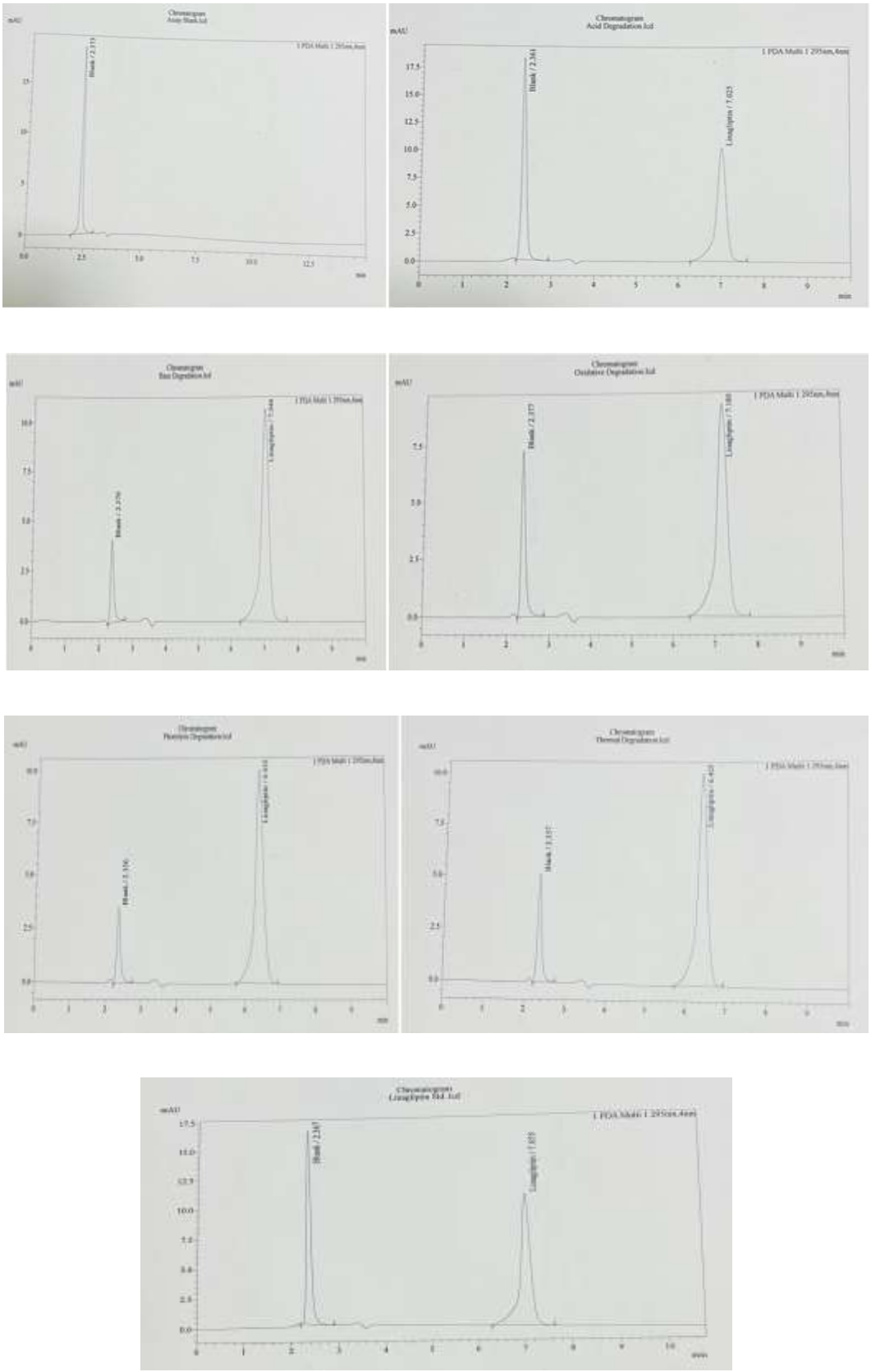
				5 mg Tablet (A-D24-L5)	306.88	163434	98.14	
40.09	165153	Basic	24th hour	API (B- D24-API)	40.92	170805	99.72	99.39
					40.34	167263	99.06	
				Linagliptin-5 mg Tablet (A-B24-L5)	301.85	163344	99.72	100.35
					306.88	168167	100.98	
40.09	165153	Oxidative	24th hour	API (O-D24-API)	40.92	167683	97.90	98.31
					40.34	166683	98.72	
				Linagliptin-5 mg Tablet (O-D24-L5)	301.85	159833	97.57	97.61
					306.88	162619	97.65	
40.05	171828	Thermal	Day-5	API (T-D5-API)	41.84	176620	96.92	96.78
					41.69	175477	96.64	
				Linagliptin-5 mg Tablet (T-D5-L5)	96.98	170599	96.98	96.82
					96.65	170807	96.65	
40.08	171686	Photolytic	Day-5	API (A-D5-API)	41.74	174858	96.25	96.62
					41.21	173947	96.98	
				Linagliptin-5 mg Tablet (P-D5-L5)	311.25	168671	96.03	96.38

4.5 Percentage degradation summary

Table 4.5: Summary of percentage degradation of the samples in stress conditions.

Samples	Assay on Day 0:	Stress conditions	% Degradation on		
			1st hour	10th hour	24th hour
API	100.85%	Acidic	0.12	0.75	1.46
		Basic	0.17	0.16	1.45
		Oxidative	0.88	1.58	2.54
Liglip-5	103.37%	Acidic	1.01	2.19	5.07
		Basic	0.89	1.53	3.02
		Oxidative	0.72	2.54	5.76

Samples	Assay on Day 0:	Stress conditions	% Degradation on		
			Day 1	Day 3	Day 5
API	100.85%	Thermal	1.41	2.74	4.07
		Photolytic	0.97	2.31	4.23
Liglip-5	103.37%	Thermal	4.44	6.27	6.55
		Photolytic	4.45	5.04	6.99



5 DISCUSSION

On 1st hour, API degraded is lowest in Acidic Degradation and higher in oxidative

degradation. Linagliptin-5mg tablets degrade lower in oxidative degradation and higher in the acidic degradation.

On 10th hour, API degraded is lowest in Basic Degradation and higher in oxidative degradation. Linagliptin-5mg tablets degrade lower in basic degradation and higher in the oxidative degradation.

On 24th hour, API degraded is lowest in Basic Degradation and higher in oxidative degradation. Linagliptin-5mg tablets degrade lower in basic degradation and higher in the oxidative degradation.

On 24th hour, API degraded is lowest in Basic Degradation and higher in oxidative degradation. Linagliptin-5mg tablets degrade lower in basic degradation and higher in the oxidative degradation.

On Day first, API degraded is lowest in photolytic Degradation and higher in Thermal degradation. Linagliptin-5mg tablets degrade lower in Thermal degradation and higher in the photolytic degradation.

On Day third, API degraded is lowest in photolytic Degradation and higher in Thermal degradation. Linagliptin-5mg tablets degrade lower in Thermal degradation and higher in the photolytic degradation.

On Day Fifth, API degraded is lowest in thermal Degradation and higher in photolytic degradation. Linagliptin-5mg tablets degrade lower in Thermal degradation and higher in the photolytic degradation.

From above results Linagliptin API and Linagliptin-5mg Tablets degrade highly in oxidative degradation with respect to Acidic and Basic degradation. API and Linagliptin- 5mg Tablets highly degrades on Photolytic degradation in comparison to thermal degradation.

There were no any degradants products formed during the study. No samples crossed the degradation limit (>20%). Thus, all samples passed the degradation studies and can be proved pharmaceutically stable in all stress conditions recommended by ICH guidelines.

6 CONCLUSION

A simple, validated, accurate, precise and analytical RP-HPLC method was selected for the

study of the drug substance and drug products. All samples were stable while applying the different stress condition i.e. Acidic, Basic, Oxidative Thermal and Photolytic degradations. In Raw material API shows highest degradation in oxidative degradation in comparison to Acid and Basic Degradation. In Comparison to thermal degradation the API and drug product tablet dosage form shows high degradation to photolytic degradation. The degradation study indicates that Linagliptin tablets have a satisfactory stability profile under recommended storage conditions. However, careful consideration of environmental factors and appropriate packaging can significantly improve the product's shelf-life and efficacy. These conclusions and recommendations help guide the appropriate handling, storage, and formulation adjustments needed to ensure the efficacy and longevity of Linagliptin tablets. Overall, the samples passed thedegradation study criteria's as per the ICH Q1A (R2), guidelines.

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