FORCED DEGRADATION STUDY OF THREE COMBINATION DRUGS REMOGLIFLOZIN ETABONATE, METFORMINE AND VILDAGLIPTIN BY RP-HPLC TECHNIQUE

Tabassum Bano* [1], Madhuri Channawar[1], AV Chandewar[1], Nitin Kocher[1], Deepak Mohale[1]

[1]P Wadhwani College of Pharmacy Dhamangaon Road Yavatmal, Maharashtra, 445001, India

***Corresponding author:**

Tabassum Bano
P Wadhwani College of Pharmacy
Dhamangaon Road Yavatmal, Maharashtra, 445001, India
Email-tabassumald@gmail.com
tabassum.bano@ghru.edu.in
Contact: 7011790073

Declarations:

Ethics approval and consent to participate: Not applicable

Consent for publication: All co-authors have given consent for the submission.

Availability of data and material: Not applicable

Competing interests: Not applicable

Funding: Not applicable

Authors' contributions: TB performed the experiments and data analysis and wrote the manuscript. MC, AVC, and NK helped with the data analysis and contributed to the correction of the manuscript. DM contributed to the correction of the manuscript. All authors reviewed the manuscript."

Acknowledgements: We wish to manifest our gratitude to Dr. A.V. Chandewar, Principal, P Wadhwani College of Pharmacy, Yavatmal, for their meritorious, inspiration, platform and continuous support for the accomplishment of this study.

Authors' information:

Tabassum Bano

Tabassum.bano@ghru.edu.in
P Wadhwani College of Pharmacy, Yavatmal, Maharashtra.

Dr. Madhuri Channawar
mchannawar@yahoo.in
P Wadhwani College of Pharmacy, Yavatmal, Maharashtra.

Dr. AV Chandewar
avchandewar@rediffmail.com
P Wadhwani College of Pharmacy, Yavatmal,Maharashtra.

Dr. Nitin Kochar
Nitin.kochar@gmail.com
P Wadhwani College of Pharmacy, Yavatmal,Maharashtra.

Dr. Deepak Mohale
Dipak.mohale@gmail.com
P Wadhwani College of Pharmacy, Yavatmal,Maharashtra.

ABSTRACT:

BACKGROUND

Forced degradation studies are a crucial tool in pharmaceutical research and development to speculate long-term stability. Stress studies should be performed in method development to know drug behavior but can also be performed with method validation for regulatory filling speculate stability, and measure impurities. It is especially useful when little data is free about potential degradation products. Therefore here we are using different regulatory guidelines in the present research paper.

RESULTS

The forced degradation studies using HILIC technique revealed the possible degradation of selected drugs; Remogliflozin, Vildagliptin and Metformin under the given stress conditions including effects of acid 0.1N Hydrochloric acid (HCl) alkali, 0.1 N Sodium hydroxide (NaOH), peroxide 3% (H_2O_2), and thermal condition (45-60˚C). As observed, upon exposure to thermal degradation at 45-60°C, both Remogliflozin and metformin were degraded where Vildagliptin was stable throughout the analysis. Furthermore, under the stress condition of 0.1N HCl Remogliflozin and Metformin have shown incurred the degradation but the Vildagliptin was stable. Furthermore, the treatment under 0.1N NaOH has made unpredictable results since as shown both pharmaceutical amines; Vildagliptin and metformin were completely disappeared and eluted with the void volume where in all circumstances Remogliflozin was stables. However, the degradation

mechanism is uncertain but it is presumed to be the involvement of protonation/deprotonation under the influence of 0.1N HCl and 20Mm ammonium acetate in mobile phase has induce such variation in retention of both Vildagliptin and Metformin. As resulted all drugs were stable since no degradants were observed. It represents, all three drugs do not have antioxidant properties.

CONCLUSION

The objective of any master plan used for forced degradation is to contribute the specified quantity of degradation, i.e., 5-20%. In this research work the drug degradation studies was found under the limit for Metformin, Vildagliptin and Remogliflozin in Acidic, basic, thermal and oxidative conditions.

KEYWORDS: Degradants, Forced degradation, Stability, Metformin, Remogliflozin, Vildagliptin.

BACKGROUND

Chemical stability of pharmaceutical molecules is an important factor as it affects the safety and efficacy of the drug product.[1] The FDA and ICH guidance's state the requisite of stability testing data to accept how the quality of a drug substance and drug product convert with time under the influence of various environmental factors. [2-6]

Aim of forced degradation studies:

Forced degradation studies are carried out to accomplish the following purposes:

1. To develop degradation pathways of drug substances and drug products.
2. To distinguish degradation products that is linked to drug products from those that are created from non-drug product in a formulation.[7-8]
3. To interpret the structure of degradation products.
4. To find out the intrinsic stability of a drug substance in formulation.
5. To release the degeneration mechanisms such as hydrolysis, oxidation, thermolysis or photolysis of the drug substance and drug product. [9-12]
6. To manifest stability indicating nature of a developed method.
7. To acknowledge the chemical properties of drug molecules.
8. To produce more stable formulations.
9. To establish a degradation profile similar to that of what would be observed in a formal stability study under ICH conditions.

10. To solve stability-related problems. [13-18]

Remogliflozin Etabonate is an anti-diabetic drug, chemically known as 5-Methyl4-[4-(1-methyl ethoxy) benzyl]-1-(1 methylethyl)- 1H-pyrazol-3-yl-6-O-(ethoxy carbonyl)-β-D glucopyranoside. It inhibits the sodium-glucose transport proteins (SGLT), which are responsible - for glucose reabsorption in the kidney. Blocking this transporter causes blood glucose to be eliminated through the urine.[19-20]

The treatment of diabetes are complicated and tedious method; hence, a multiple intervention approach such as the practice of healthy diets, physical activity, and various therapeutic strategies may help to minimize the complications of diabetes. Dipeptide peptidase-4 (DPP-4) and sodium-glucose transporter-2 (SGLT-2) inhibitors showed an enhanced HbA1c control when compared with conventional sulfonylureas and thia-zolidinediones [21]. The Food and Drug Administration (FDA) has approved a fixed-dose Remogliflozin and Vildagliptin tablet for T2DM. Remogliflozin etabonate (RGE) is an oral hypoglycemic drug [22], which acts by inhibiting the SGLT-2 enzyme and thereby decreasing the reabsorption of glucose from the glomerular filtrate back to the blood.

SGLT-2 inhibitors reduce cardiovascular events, body weight, and also show a defensive effect on the renal system. These functional properties of SGLT-2 inhibitors considerably reduce the hospitalization of T2DM patients exclusively due to heart failure [23-24]. Vildagliptin (VGN), a DPP-4 inhibitor, decreases the blood sugar level by protecting the incretins from degradation, which helps in the production of insulin after food and reduces glucagon formation in the liver. The protection of incretins also helps in reducing body weight by decreasing the appetite and prolonging the slow digestion of food [25, 26]

EXPERIMENTAL WORK

Several attempts were made on simultaneous analysis of Metformin with either Vildagliptin or Remogliflozin but no work were carried out on simultaneous analysis of all three drugs combination. Importantly, all separation was performed using reverse phase chromatography, specifically by conventional C18 column with improved adsorbent properties. Nevertheless, there are certain drawbacks of this technique, such as first; as highly polar nature of Metformin, it elute with dead volume in RP-HPLC. Second, the Remogliflozin is moderately non-polar in nature so it retain strongly in C18

phase. Third, the Vildagliptin has very low UV sensitivity and hence it gets detected at low UV wavelength which again causes unstable base line. In addition, while considering RP-HPLC for simultaneous estimation of Metformin with Vildagliptin and Remogliflozin, the results achieved with elongated run time and resolution, and most importantly, Metformin does not retain in ODS phase.

Alternative to this the HILIC technique was first time utilize for this simultaneous estimation of all three selected drugs. As resulted Metformin eluted quite late and it is reverse to that of RP-HPLC whereas Remogliflozin eluted earlier with retention factor was ≤ 0.5. The total run time was shortened to 6 minutes which in results increase its separation efficiency.

Reagents and reference samples

The reference standards; Metformin, Remogliflozin and Vildagliptin were obtained as a gift samples from Yarrow Pharma Chem Ltd. Ammonium acetate from Merck Ltd. (Mumbai-India) HPLC grade acetonitrile and deionised water from Merck (Mumbai, India). 0.20µ and 0.45µ nylon membrane filters were used from UltraChrom Innovatives Pvt. Ltd. (India). All other chemicals and reagents were used of HPLC grade.

Standard stock solutions

Standard stock solutions of 1 mg/mL of standards, Metformin, Remogliflozin and Vildagliptin were prepared separately by dissolving 10 mg of the drug in 10 ml of Acetonitrile: Methanol: Water (3:4:3 v/v) in a 20 mL volumetric flask. Furthermore, freshly prepared standards were mixed together to get the concentration 100 ppm each for performing validation studies like repeatability, precision and robustness studies. Standard stock solution was then ultrasonicated for 10-20 minutes and filtered through 0.20µ nylon filters prior to the HPLC analysis.

Chromatographic conditions

Chromatographic separation was achieved on Acclaimed Mix Mode HILIC-1 column (150 mm × 4.6 mm, 5µm) applying an isocratic elution based on 20 mM ammonium acetate : acetonitrile (75:25, *v/v*) as a mobile phase. The ultraviolet detector was operated at 230 and 254 nm. The buffer solution was filtered through 0.2 µm nylon membrane filter and degassed for 10-20 min in an ultrasonic bath prior to its use. The mobile phase was pumped through the column at a flow rate of 1 mL min^{-1}. The column temperature was adjusted to 28°C and the injection volume was 20 µL.

The forced degradation studies using HILIC technique revealed the possible degradation of selected drugs; Remogliflozin, Vildagliptin and Metformin under the given stress conditions including effects of acid (0.1N HCl) alkali (0.1 N NaOH), peroxide (3% H_2O_2), and thermal condition (45-60˚C).

Effect of Thermal degradation

As observed, upon exposure to thermal degradation at 45-60°C, both Remogliflozin and Metformin were degraded where Vildagliptin was stable throughout the analysis (FIG No. 1 and Table 1).

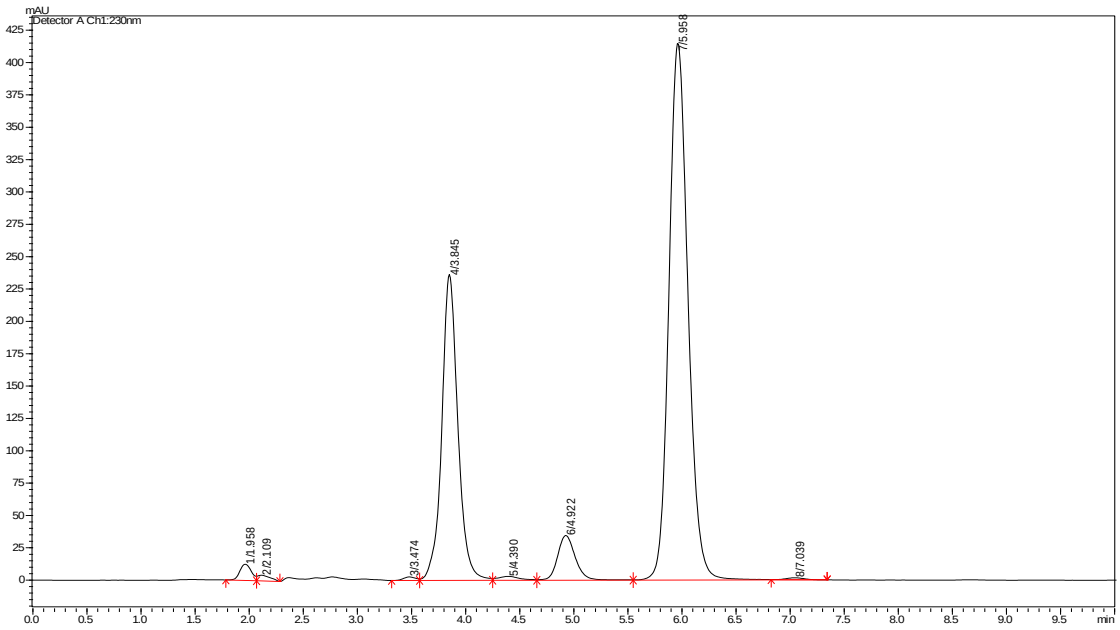

Figure 1; Effect of Thermal degradation AT 45-60°Con REM, VIL and MET

Table 1; Effect of Thermal degradation

Peak#	Ret. Time	Area	Height	Area%	T. Plate#	Resolution	k'	Tailing F.
1	1.958	93279	12532	1.1463	1407.127	--	0	--
2	2.109	34943	4401	0.4294	513.772	0.518	0.077	--
3	3.474	21855	2712	0.2686	3047.163	4.378	0.774	--
4	3.845	2444522	236367	30.0418	3280.245	1.426	0.964	1.08
5	4.39	42073	3024	0.517	2009.627	1.649	1.242	--
6	4.922	394595	34600	4.8494	4402.946	1.545	1.513	1.194
7	5.958	5088654	414614	62.5366	5449.046	3.345	2.042	1.168
8	7.039	17154	1527	0.2108	8053.632	3.397	2.594	1.111

Effect of 0.1 N HCl

Furthermore, under the stress condition of 0.1N HCl Remogliflozin and Metformin have shown incurred the degradation but the Vildagliptin was stable (FIG No.2 and Table 2).

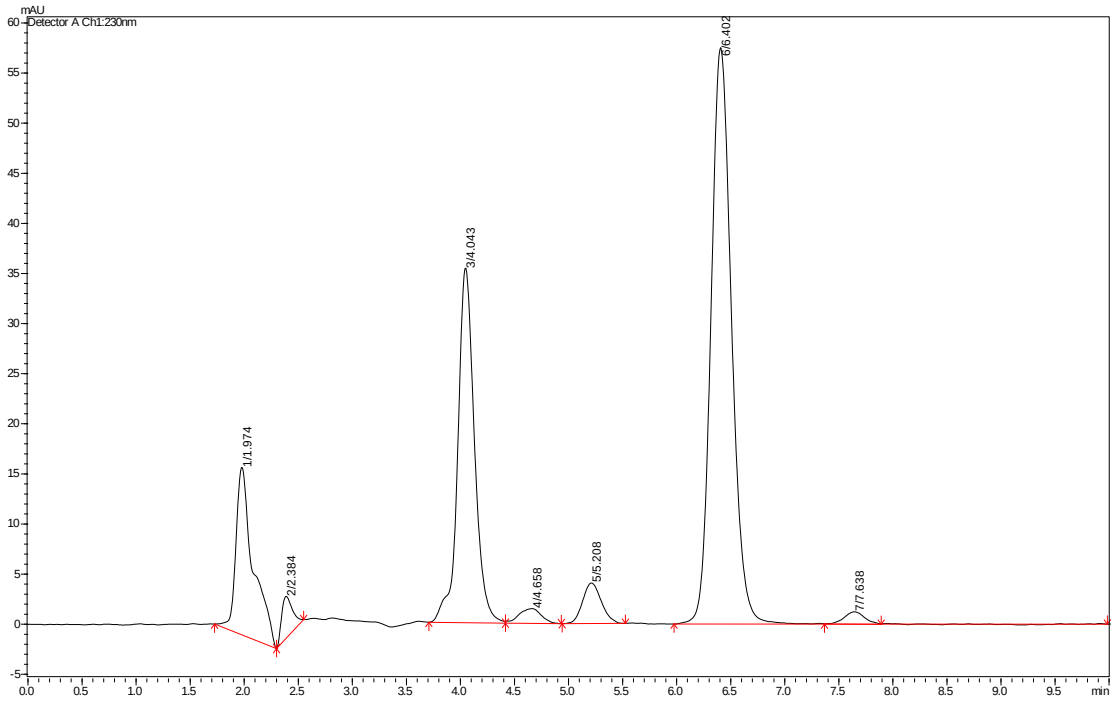

Figure 2; Effect of 0.1 N HCl at 45-60°Con REM, VIL and MET

Table 2; Effect of 0.1 N HCl at 45-60°C

Peak#	Ret. Time	Area	Height	Area%	T. Plate#	Resolution	k'	Tailing F.
1	1.974	185522	16694	12.9418	1120.577	--	0	1.696
2	2.384	30010	4236	2.0935	2355.061	1.895	0.208	1.525
3	4.043	379636	35387	26.483	3466.972	7.043	1.048	0.988
4	4.658	21071	1476	1.4699	2562.518	1.913	1.359	--
5	5.208	48822	4046	3.4058	4065.731	1.584	1.638	1.158
6	6.402	752037	57521	52.4612	5402.897	3.538	2.243	1.166
7	7.638	16412	1240	1.1449	7699.817	3.549	2.869	--

Effect of 0.1 N NaOH

Furthermore, the treatment under 0.1N NaOH has made unpredictable results since as shown both pharmaceutical amines; Vildagliptin and Metformin were completely disappeared and eluted with the void volume where in all circumstances Remogliflozin was stables (FIG No. 3 and Table 3).

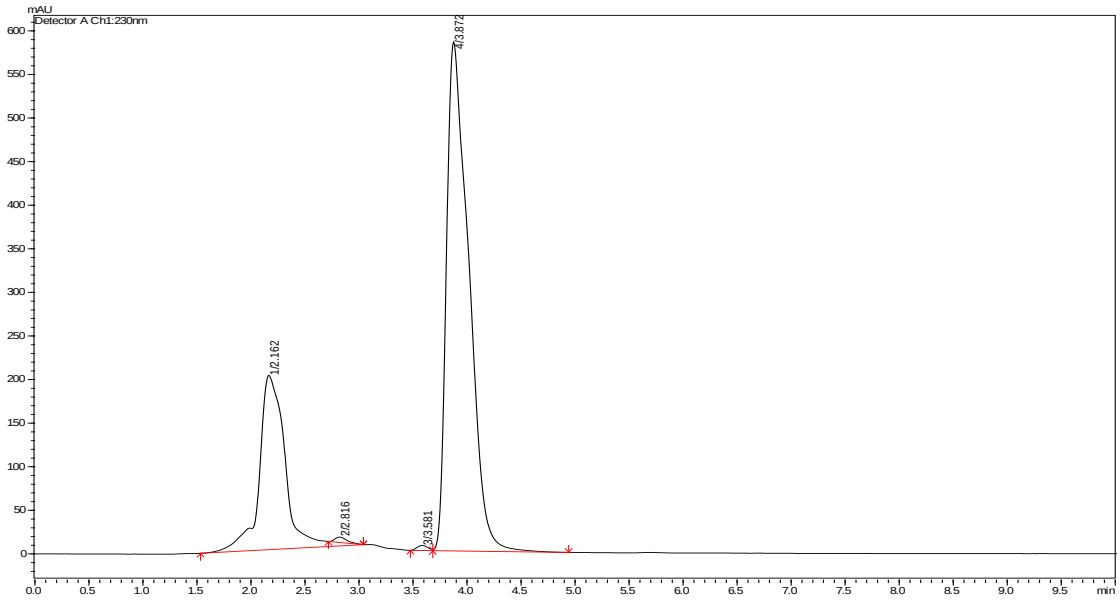

Figure 3;Effect of 0.1 N NaOH at 45-60°Con REM, VIL and MET

Table 3; Effect of 0.1 N NaOH at 45-60°C

Peak#	Ret. Time	Area	Height	Area%	T. Plate#	Resolution	k'	Tailing F.
1	2.162	3342791	200006	28.3638	569.449	--	0	1.131
2	2.816	46977	6119	0.3986	2842.672	2.279	0.302	1.625
3	3.581	40256	5725	0.3416	4971.077	3.693	0.656	--
4	3.872	8355397	584083	70.896	1495.24	0.964	0.791	1.739

Effect of 3% H_2O_2

Similarly, the treatment under 3-6% H_2O_2 has not made any significant changes in stability of all selected drugs. As resulted all drugs were stable since no degradants were observed in figure 2. It represents, all three drugs do not have antioxidant properties.

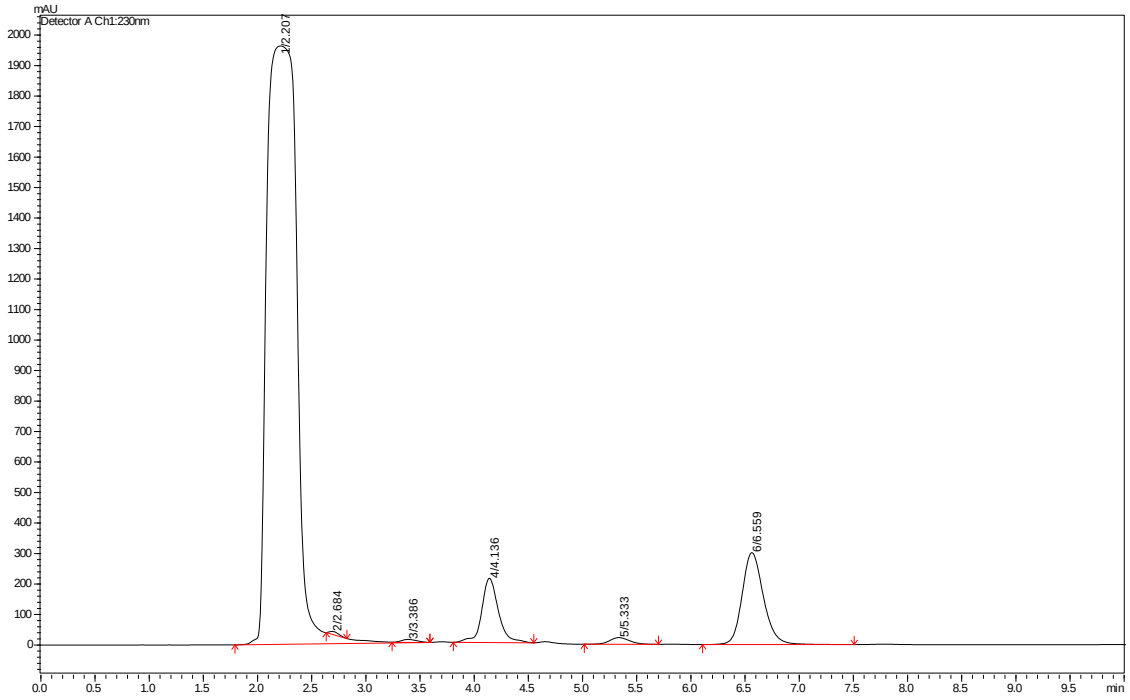

Figure 4;Effect of 3%H_2O_2 at 45-60°Con REM, VIL and MET

Table 4;Effect of 3%H_2O_2 at 45-60°C

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.
1	2.207	37471876	1961761	84.6255	498.804	--	0	1.246
2	2.684	54758	8986	0.1237	4213.102	1.704	0.216	1.785
3	3.386	91652	9297	0.207	2372.128	3.166	0.535	1.231
4	4.136	2254059	210628	5.0905	3756.598	2.734	0.874	1.038
5	5.333	280416	21932	0.6333	3915.346	3.919	1.417	1.124
6	6.559	4126900	301305	9.3201	5368.07	3.509	1.972	1.208

Force degradation studies of REM, VIL and MET

Table 5; force degradation studies of Remogliflozin

Conditions: Remogliflozin	No. of degradants (fragments)	% degradation
Acid (0.1N NaOH) + 45˚C + 12 Hrs.	1 degradant	1.46%
Base (0.1N/M HCl) + 60˚C + 12 Hrs.	----	---
Thermal (45˚C) + 12 Hrs.	2degradants	0.78%
Oxidation (6% H_2O_2) + Room Temp.	No degradation	None

Table 6; force degradation studies of Vildagliptin

Conditions: Vildagliptin	No. of degradants (fragments)	% degradation
Acid (0.1N NaOH) + 45˚C + 12 Hrs.	No degradation	----
Base (0.1N/M HCl) + 60˚C + 12 Hrs.	---	-----
Thermal (45˚C) + 12 Hrs.	No degradation	None
Oxidation (3-6% H_2O_2) + Room Temp.	No degradation	None

Table 7; Force degradation studies of Metformin

Conditions: Metformin	No. of degradants (fragments)	% degradation
Acid (0.1N NaOH) + 45˚C + 12 Hrs.	1 degradant	1.14%
Base (0.1N/M HCl) + 60˚C + 12 Hrs.	----	----
Thermal (45˚C) + 12 Hrs.	1 degradants	0.21%
Oxidation (3-6% H_2O_2) + Room Temp.	No degradation	None

However, the degradation mechanism is uncertain but it is presumed to be the involvement of protonation/deprotonation under the influence of 0.1N HCl and 20Mm ammonium acetate in mobile phase has induce such variation in retention of both Vildagliptin and Metformin. Hence, future studies involved to understand the exact chemical transformation using the same chromatographic condition by using LC-MS/MS or LC-NMR technique.

CONCLUSION:

Forced degradation studies contemplate the facts about possible degradation pathways and results of the energetic fixings and assist to elucidate the structure of degradants. Degradation products bring out from forced degradation studies i.e.likely degraded products that might not be created under specific storage conditions, but they can help in the developing stability-indicating method. The objective of any master plan used for forced degradation is to contribute the specified quantity of degradation, i.e., 5-20%. In this research work the drug degradation studies was found under the limit for Metformin, Vildagliptin and Remogliflozin in Acidic, basic, thermal and oxidative conditions.

LIST OF ABBREVIATION

FDA:Food and Drug Administration

ICH:International Council for Harmonisation

HCL:Hydrochloric Acid

H_2O_2:Hydrogen Peroxide

NAOH:Sodium Hydroxide

LC-MS:Liquid Chromatography-Mass Spectrometry

LC-NMR:Liquid Chromatography with Nuclear Magnetic Resonance Spectroscopy

REM:Remogliflozin

VIL:Vildagliptin

MET: Metformin

HPLC: High Performance Liquid Chromatography

REFERENCES

1. Blessy Mn, RuchiD.Patel, PrajeshN. Prajapati, Y.K.Agrawal(2014) Development of forced degradation and stability indicating studies of drugs. A review Journal of Pharmaceutical Analysis4(3):159–165.
2. ICH guidelines, Q1A (R2): Stability Testing of New Drug Substances and Products (revision2), International Conferenceon Harmoniza-tion. Availablefrom: ⟨http://www.fda.gov/downloads/RegulatoryInformation/Guidances/ucm128204.pdf⟩, 2003.
3. D.W. Reynolds, K.L. Facchine, J.F. Mullaney (2002) Available guidance and best practices for conducting forced degradation studies, Pharm. Technol.26(2)48–56.
4. H. Brummer (2011) How to approach a forced degradation study, Life Sci. Technol. Bull. 31:1–4.
5. ICH Harmonised Tripartite Guideline stability testing: Photostability testing of new drug substances and products Q1 B.
6. Von V, Helene J, Aus S. 2015. Forced degradation studies - comparison between ICH, EMA, FDA and WHO guidelines and ANVISA's resolution RDC 53/2015.
7. ICH and FDA: Q1A (R2) stability testing of new drug substances and products. Geneva Switzerland and Rockville MD 2003. 20.
8. ICH Topic Q1A (R2) stability testing of new drug substances and products, proceedings of the international conference on harmonization. EMEA London 2003.

9. International Conference on Harmonization (ICH) and US Food and drug Administration, Q1B: photostability testing of new drug substances and products. Geneva Switzerland and Rockville MD 1996
10. Iram F, Iram H, Iqbal AZHAR, Husain A. (2016) Forced degradation studies. J Anal Pharm Res*3*(6): 00073.
11. Bhaskar R, Ola M, Agnihotri V, Chavan A, Girase H. (2020) Current Trend in Performance of Forced Degradation Studies for Drug Substance and Drug Product's. Journal of Drug Delivery and Therapeutics 15; 10(2-s): 149-55.
12. Singh S, Junwal M, Modhe G, Tiwari H, Kurmi M, Parashar N, Sidduri P.(2013) Forced degradation studies to assess the stability of drugs and products. TrAC Trends in Analytical Chemistry 1; 49: 71-88.
13. EMEA C. Guideline on the limits of genotoxic impurities. Committee for Medicinal Products for Human Use (CPMP). 2006.
14. Venkataraman S, Manasa M. (2018) Forced degradation studies: Regulatory guidance, characterization of drugs, and their degradation products-a review. Drug Invention 1; 10(2).
15. Yasmeen A, Sofi G. A (2019) review of regulatory guidelines on stability studies. The Journal of hytopharmacology8: 147-51.
16. Naveed S, Basheer S, Qamar F (2016) Stability of a dosage form and forced degradation studies. J Bioequivalence Bioavailab 8: 191-3.
17. Oyler AR, Segmuller BE, Sun Y, (2012)forced degradation studies of rapamycin: Identification of autoxidation products. J Pharm Biomed Anal59:194–200.
18. Trabelsi H, Hassen IE, Bouabdallah S, (2018) Stability indicating LC method for determination.Russian Original 52: 4.
19. Sami W, Ansari T, Butt NS, Hamid MRA, (2017) Effect of diet on type 2 diabetes mellitus: A Review. Int. J. Health Sci. 11: 65–71.
20. Magliano DJ, Sacre JW, Harding JL, Gregg EW, Zimmet PZ, Shaw JE,(2020)Young-onset type 2 diabetes mellitus—Implications for morbidity and mortality. Nat. Rev. Endocrinal 16: 21–31.
21. Moon MK, Hur KY, Ko SH, Park SO, Lee BW, Kim JH, Rhee SY, Kim H, Choi, (2017)Insulin Therapy for Adult Patients with Type 2 Diabetes Mellitus. A

Position Statement of the Korean Diabetes Association. Diabetes Metab J. 41(5):367-373.

22. KM Kim NH, (2017) Committee of Clinical Practice Guidelines of the Korean Diabetes Association. Combination therapy of oral hypoglycemic agents in patients with type 2 diabetes mellitus. Korean J. Intern. Med.32: 974–983.
23. Rena G, Hardie DG, Pearson ER, (2017) The mechanisms of action of metformin. Diabetologia 60: 1577–1585.
24. Mohan V, Mithal A, Joshi SR, Aravind SR, Chowdhury S, (2020) Remogliflozin Etabonate in the Treatment of Type 2 Diabetes: Design, Development, and Place in Therapy. Drug Des. Dev. Ther. 14: 2487–2501.
25. Markham A,(2019) Remogliflozin Etabonate: First global approval. Drugs 79: 1157–1161.
26. Simes BC, MacGregor GG, (2019) Sodium-Glucose Cotransporter-2 (SGLT2) Inhibitors: AClinician's Guide. Diabetes Metab. Syndr. Obes. 12: 2125–2136.