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FORCED DEGRADATION STUDY OF ROSUVASTATIN AND TENELIGLIPTIN, CHARACTERISATION OF ITS DEGRADATION PRODUCTS BY VARIOUS ANALYTICAL TECHNIQUES: A REVIEW

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ABSTRACT: Rosuvastatin is a widely used lipid-lowering medication that belongs to the class of "Statins" whereas Teneligiptin is a novel potent drug that belongs to the class of "DPP-4 inhibitors" which is used for the treatment of Type 2 Diabetes mellitus. The FDA recently approved the combination of these two drugs in 2021 since it proved to be highly effective for treating dyslipidemia associated with Type 2 Diabetes mellitus. Forced degradation studies have been performed on Rosuvastatin and Teneligliptin separately using various analytical techniques such as RP-HPLC, UV, RP-UFLC, RP-UPLC, UHPLC, HPTLC and TLC. Characterization of the degradation products generated during various stress conditions is also performed using various sophisticated techniques such as UPLC, LC/MS/MS, NMR, and FT-IR. These degradants can cause many adverse effects, such as carcinogenicity, mutagenicity, neurotoxicity, hepatotoxicity, skin sensitization, respiratory sensitization, *etc*. So, identifying and characterizing these degradation products will be very useful for their metabolic studies and *in-silico* toxicity assessment. *In-silico* toxicity studies are very helpful in predicting the toxic potential of these degradants, and it is performed using various software such as TOPKAT, Osiris, and DEREK. Thus, the present review aims to summarise the forced degradation studies as well as characterization of the degradation products generated from both rosuvastatin and teneligliptin using various modern analytical techniques so that it will significantly contribute in the future regarding their metabolic studies, determination of impurities during their bulk synthesis as well as toxicity predictions.

INTRODUCTION: Degradation can be defined as the incapability of a particular substance to remain within its particular physical, chemical, microbiological, toxicological, and therapeutic specifications. "Forced degradation can be defined as the degradation of a New drug substance or new drug product at conditions more severe than the accelerated conditions.

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Forced degradation studies depend on the type of product and the dosage form. It is mandatory to establish the specificity of the stability indicating methods and provide insight into degradation pathways and degradation products of the drug substance that are likely to be formed" [1]. It also helps in the structure elucidation of the degradation products.

According to FDA and ICH, stress testing is necessary to understand how the quality of a drug substance and product changes with time under the influence of various environmental factors. Felicitous knowledge about the molecule's stability helps in selecting proper formulation and package and provide proper storage conditions and shelf

life, which are essential for regulatory documentation. Degradation products generated during these testing can be studied to determine the molecule's stability. "The stability studies include long-term studies (12 months) and accelerated stability studies (6 months).

But intermediate studies (6 months) can be performed at conditions milder than that used in accelerated studies. So the study of degradation products like separation, identification, and quantitation would take even more time. Compared to stability studies, forced degradation studies help generate degradants in a much shorter span of time, mostly a few weeks.

Objective of Forced Degradation Studies:

1. To establish degradation pathways of drug substances and drug products.
2. To differentiate degradation products related to drug products from those generated from non-drug products in a formulation.
3. To elucidate the structure of degradation products.
4. To determine the intrinsic stability of a drug substance in the formulation.
5. To reveal the degradation mechanisms such as hydrolysis, oxidation, thermolysis or photolysis of the drug substance and product.
6. To establish stability indicating the nature of a developed method.
7. To understand the chemical properties of drug molecules.
8. To generate more stable formulations.
9. To produce a degradation profile similar to what would be observed in a formal stability study under ICH conditions.
10. To solve stability-related problems.

Degradation of drug substances between 5% and 20% have been accepted as reasonable for validating chromatographic assays. It is recommended that the studies should be initiated at a concentration of 1 mg/mL. By using a drug concentration of 1 mg/mL, it is usually possible to get even minor decomposition products in the range of detection" [1].

A combination of Rosuvastatin and Teneligliptin treats Type 2 DM and Dyslipidemias. Rosuvastatin (depicted in **Fig. 1**) belongs to a bunch of medication called "Statins", which decreases LDL and Triglycerides and lift HDL within the blood. "It competitively inhibits the conversion of 3-hydroxy-3-methyl glutaryl coenzyme A to mevalonate (rate-limiting step in CH synthesis) by the enzyme HMG CoA reductase. Therapeutic dose reduces CH synthesis by 20-50%. This results in a compensatory increase in LDL receptor expression on liver cells – increased receptor-mediated uptake and catabolism of IDL and LDL. All statins except rosuvastatin are metabolized primarily by CYP3A4. In patients with raised TG levels, rosuvastatin raises HDL-CH by 15 – 20%" [2]. Teneligliptin (depicted in **Fig. 2**) is an antidiabetic medication that decreases blood glucose levels by enhancing insulin release from the pancreas. Thereby fasting and post-meal sugar level reduces.

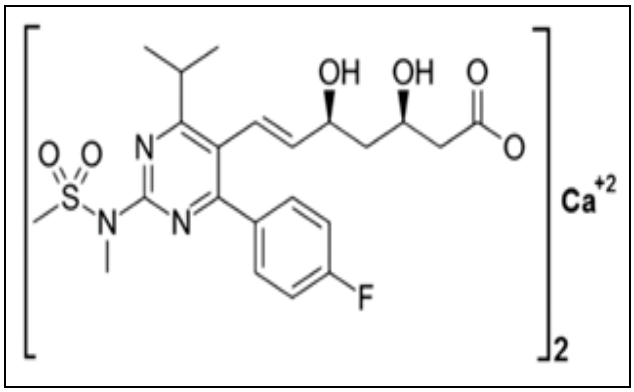

FIG. 1: ROSUVASTATIN CALCIUM: (E)-(3R, 5S)-7-{4-(4 - FLOUROPHENYL) – 6 – ISOPROPYL – 2 - [METHYL (SULPHONYLAMINO)] PYRIMIDIN-5-YL}-3,5- DIHYDROXYHEPTEN-6-OIC ACID CALCIUM

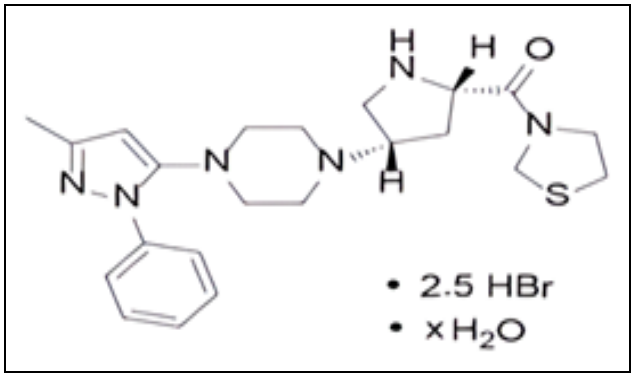

FIG. 2: TENELIGLIPTIN HYDROBROMIDE HYDRATE: {(2S, 4S)-4-[4-(3-METHYL-1-PHENYL-1H-PYRAZOL-5-YL) PIPERAZINE-1-YL] PYRROLIDINE-2-YL} (1, 3 - THIAZOLIDINE – 3 - YL) METHANONE HEMIPENTAHYDROBROMIDE HYDRATE

"Diabetes and increasing resistance to insulin, even in persons considered to have ''normal'' insulin sensitivity, has been associated with higher concentrations of cholesterol, LDL and TG and lower concentrations of HDL cholesterol [3, 4], which results in "Atherogenic Dyslipidemia" and worsens the prognosis of diabetic patients by synergistically accelerating atherosclerosis and development of CVD. It has been hypothesized that most oral anti-diabetic drugs have significant lipid lowering effect besides achieving effective glycaemic control. Thus, they can modify dyslipidemia and help decrease the risk of atherosclerosis, coronary heart disease, stroke, nephropathy and nephropathy and retinopathy" [5].

In patients with diabetes, adherence to statin therapy was poor, with a reduction from 87% in starting 3 months to less than 50% 6 months onwards. Through the combination, it has been proven to improve medication when compared to its free drug component treatment. There is also a remarkable reduction in HbA1c, a reduction in Low-density lipoprotein (LDL) level at 24 weeks, and a decreases threat of heart disease in diabetic patients [6]. While going through the literature survey, it was found that no forced degradation studies have been done in this drug combination. Also, characterization of the degradation products generated during various stress conditions is not performed till now. This review aims to provide updated information about forced degradation studies done in this drug combination using various analytical techniques and characterization of degradation products which will be useful in the future for conducting *in-silico* toxicity studies.

METHODS:

Characterization of Degradation Products: Identifying the degraded products helps in future metabolic studies and related impurity determination during its bulk synthesis. Characterization can be done using various modern analytical techniques such as:

- UPLC
- LC/MS/MS
- NMR
- FT-IR

Various types of forced degradation studies on drug substances and products are depicted in **Fig. 3** [7].

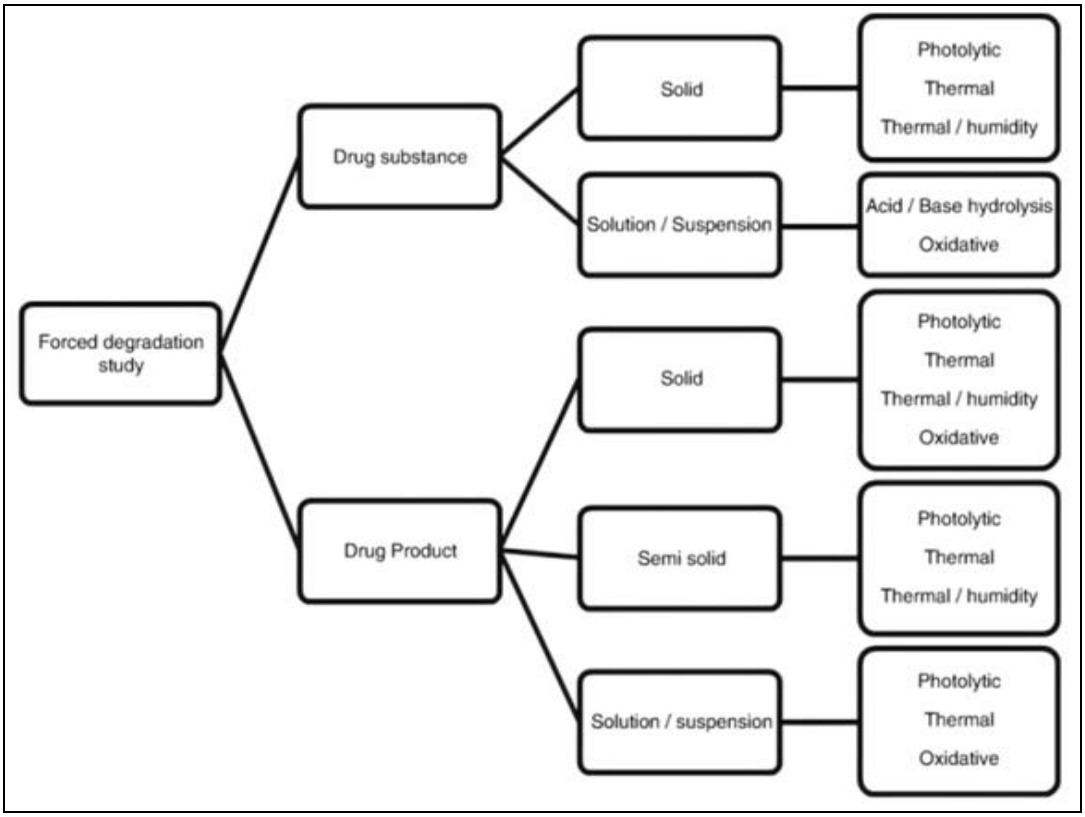

FIG. 3: FORCED DEGRADATION STUDY OF DRUG SUBSTANCE AND DRUG PRODUCT

Forced Degradation Study of Rosuvastatin using Various Analytical Techniques: The optimum conditions for performing forced degradation studies are summarised in **Table 1, Table 2, Table 3** and **Table 4.**

TABLE 1: FORCED DEGRADATION STUDIES USING HPLC

Sl. no.	Acid Hydrolysis	Alkaline Hydrolysis	Neutral Hydrolysis	Oxidation	Thermal Hydrolysis	Photolysis	Ref.
1	0.1N HCl, 80ºC, 1 hr	2N NaOH, 80 ºC , 48 hr	Water, 48hr, 80 ºC	30% H_2O_2, RT, 48 hr	50 ºC, 21 days	8,500 lux fluorescent, ~ 0.5 W/m2 UV	8
2	0.1 – 1M HCl/H_2SO_4, 50 - 70 ºC, 7 days	0.1 – 1M NaOH/KOH, 50 - 70 ºC, 7 days		0.1- 3% H_2O_2 ,neutral pH, 7 days	40 – 80ºC	1.2 million lux hours, 200 Wh/m2 light.	9
3	0.2M HCl, 80ºC, 20 hr	Borate buffer 1 N NaOH 80ºC	6.8 pH buffer,80ºC, 20 hr	0.5 % H_2O_2 80ºC, 20 hr	100 ºC,24 hrs,121ºC, 15lb, 20min	1.2 * 10^9 lux/hr	10
4	0.1N HCl, 80 ºC,30min	0.1NaOH, 80 ºC, 30 min		30% H_2O_2, 80 ºC, 30min	80 ºC, 10 hr	UV-365nm 3hrs	11
5	0.5N HCl, 72 hrs and 1N HCl, 5 hrs, 80ºC reflux	1 N NaOH, 48 hrs and 4 hrs,80ºC reflux	Water, 48 hrs and 3hr 80ºC reflux	30% H_2O_2, 48 hrs. and 5 hrs, 80ºC reflux	80ºC, 48 hrs	UV–254nm Vis–366nm 48 hrs	12
8	0.01N HCl, 60 ºC,30min reflux	0.01N NaOH, 40 ºC, 10 min reflux	Water, RT, 24 hrs	10% H_2O_2 60ºC, 10 min reflux	105ºC, 24 hrs		13
9	0.1N HCl 80 ºC,24 hrs, 4 hrs and 2 hrs refllux and 2 days, 50ºC	0.1N NaOH, 80 ºC,24 hr, 4 hrs and 2 hrs and 2 days,50 ºC	80ºC, 24 hrs and 4 hrs	30%H_2O_2, 24 hrs and 2 days, 50ºC 3% H_2O_2,80 ºC, 4 hrs and 6% H_2O_2 2 hr, reflux	Sandbath, 50ºC,24 hrs and 105 ºC, week, 70ºC, 48 hrs and 2 days, 50ºC	Sunlight, 30 days,48 hrs and 1.2 million lux hrs+ UV of 200Wh/m^2	14 15, 16 17 18
10	0.1N HCl, 60 ºC, 2 hrs	5N NaOH, 60 ºC, 12hrs	Water, 60ºC, 30 min	10% H_2O_2 60ºC, 30 min	105ºC, 6 hrs	Visible, UV- 1.2 mllion lux hrs and 200 Wh/m^2	19 20

Discussion and Interpretation: For the degradation study done by authors of reference [8], the drug was found to be unstable under acid hydrolysis and photolysis. It was stable under basic, neutral hydrolysis, oxidation and thermal conditions. Here, eleven degradation products were formed. Five degradation products were formed under acid hydrolysis – RS-1, RS-6, RS – 7, RS – 10, RS-11. Six degradation products were formed under photolysis conditions – RS-2, RS – 3, RS – 8, RS – 9, RS – 4, RS – 5. Merit of this work was RS-2, RS-3, RS-6, RS-10 and RS-11 are previouly unknown degradation products of rosuvastatin, got detailed information about these products. Structure elucidations of all the degradation products were done using LC-MS/TOF, LC-MS^n, on-line H/D exchange and LC-NMR. MS/TOF studies were performed in ESI positive mode. The fragmentation pathway of drug under various stress conditions, characterisation of the formed degraded products using various techniques along with their *in-silico* toxicity assessment was well explained. H^1 NMR and COSY spectra were recorded using the WET pulse sequences. *In-silico* toxicity of all degradation products was predicted using TOPKAT and DEREK software.

For reference [9], the merit of this work was that the objectives, time to perform degradation, selection of drug concentration, and limits for degradation are well explained. The demerit associated with this work is that the procedure for photolysis and thermal degradation is not well explained

Reference [10] found that the drug was stable under neutral and alkaline hydrolysis. Unstable under acidic, photolytic, and oxidation conditions. Photolytic degradation is observed to be very prominent. The demerit of this work was that the neutral hydrolysis is performed using phosphate

buffer, as well as characterization of degraded products is not done. Merit of the present work is that, this method is very specific and can be used to determine rosuvastatin calcium in the presence of its various degradation products and excipients used in the tablet form.

Reference [11], rosuvastatin has significantly undergone alkaline degradation due to the presence of carboxylic acid group in its structure. It is slightly sensitive toward photolytic degradation. Degree of degradation is very less under acidic, oxidation and thermal degradation. The advantage of this work was that it very useful for the long term stability studies of pharmaceutical dosage forms. The disadvantage is that degraded products generated under various stress conditions are poorly explained.

Reference [12] shows the drug was highly unstable under acidic, oxidation, and photolytic conditions. Slightly sensitive to thermal degradation. It is comparatively stable in neutral and alkaline conditions. Seven degradation products were formed as a part of degradation studies. The merit of this work was that the degree of degradation under various stress conditions is well explained.

Reference [13], here, drug is highly unstable under thermal thermal degradation (20%) and acidic hydrolysis (16%). It was found to be stable under neutral hydrolysis. The advantage of the present work is that this method can be used to determine Rosuvastatin and Ezetimibe in the capsule dosage form. The disadvantage found in this work is that photolytic degradation is not performed.

Reference [14], major degradation of the drug has occurred under acidic hydrolysis (57%), 36% under alkaline conditions, 32% under oxidation, 30% under photolytic degradation, and 15% under thermal degradation. The main advantage of this work was that YMC packs C8 column was used, having several advantages like high resolving power, low tailing *etc*. Demerit of the present work is that the number of degradation products generated from each stress condition is not specified.

Reference [15] shows the drug's major degradation occurred under alkaline hydrolysis (12.9%). No degradation was found under oxidation and thermal conditions. The merit of this work is that this method explains the degradation characteristics of Rosuvastatin calcium and Fenofibrate in the combined dosage form. The main demerit is the long duration for all stress degradation studies (24 hours).

Reference [16], the drug was found to be highly degraded under acidic hydrolysis and oxidation conditions by forming two degraded products. In thermal degradation, the drug was found to be highly stable. The merit of this work is that, this method can be applied for the routine quality control analysis of Rosuvastatin calcium in bulk and tablet dosage form. Demerit is that the number of degradation products formed under each stress condition is not specified.

Reference [17] shows that rosuvastatin was unstable under acidic, alkaline, photolytic, and oxidation conditions. Major degradation has occurred under acidic hydrolysis (35.7%). Metformin was decomposed only under alkaline hydrolysis. The advantage of the present work is that this method can be used for simultaneous quantitation of Rosuvastatin and Metformin in a combined oral solid dosage form. The main disadvantage was that the thermal degradation duration is very long (1 week).

Reference [18], drug was found to be unstable under acidic, alkaline, oxidation, and thermal degradation. Two degradation products were formed under alkaline hydrolysis. 94% of the drug has degraded under oxidation conditions in the presence of 30% H_2O_2. The advantages of the present work are that it is cost-effective, a green mobile phase has been used, and speed of analysis. Disadvantages include very long duration for all forced degradation studies (2 days) and the degradation under neutral hydrolysis and photolysis condition is not performed.

Reference [19], drug was significantly degraded under acidic hydrolysis, oxidation and photolysis. New impurity was not generated during acidic, basic, neutral hydrolysis or under oxidation, photolysis, or humidity degradation. Only in thermal degradation 0.18% of the impurity was generated.

In this study, a new impurity of rosuvastatin was found during the analysis of accelerated stability samples by HPLC. Identification of impurity is done by LC-MS/MS using an Electrospray ionisation source and Q trap mass analyzer.

Based on the analysis, it was found that condensed product of Meglumine excipient with Rosuvastatin molecule was generated as the impurity. Characterization was done using LC-MS, HRMS (UPLC-TOF-MS), NMR and FT-IR spectroscopy. In NMR spectroscopy, both H1 and C13 NMR spectra were recorded.

In reference 20, the drug's Anti – isomer and lactone impurities were generated in acidic hydrolysis. In photolysis, two unknown impurities are formed. In thermal degradation, one unknown impurity was generated. The merit of this work is that the impurities were characterized using ESI – MS, HRMS, and NMR spectroscopic methods. ESI mass spectrum of Rosuvastatin impurity was recorded on Q trap LC-MS/MS system.

Mass spectral data indicate the formation of the condensed product of Meglumine with Rosuvastatin. HRMS spectrum confirms the molecular formula of the impurity as $C_{29}H_{44}N_40_{10}FS$.

Recommendation: Among all the forced degradation studies done using the RP-HPLC technique, it is seen that references [8, 19, 20] are better when compared with the remaining references because only in these works, characterization of the degradation products or impurities generated during various stress conditions is performed using sophisticated techniques such as LC-MS/MS, HRMS, NMR, and FTIR spectroscopy.

Among these three references, reference [8] can be considered the best and will be very useful in the future, because only in this work is the in-silico toxicity assessment of the degradation products well explained using software such as TOPKAT and DEREK.

TABLE 2: FORCED DEGRADATION STUDIES USING UPLC

Sl. no.	Acid Hydrolysis	Alkaline Hydrolysis	Neutral Hydrolysis	Oxidation	Thermal Hydrolysis	Photolysis	Ref.
1	0.1 N HCl, 80 ºC, 2 hr	0.5N NaOH 80 ºC, 6 hr		3% H_2O_2, 80 ºC, 6 hr	100ºC, 8 hr	UV light	[21]
2	1 N HCl, 3hrs, RT	1 N NaOH, 70ºC, 2 hrs		5% H_2O_2, 70ºC, 2 hrs	105ºC, 7 days	1.2 million lux hours-200Wh/m^2 7 days	[22]

Reference [21] revealed four unknown degradation products when exposed to acidic conditions. The drug was relatively stable when exposed to basic, thermal, and oxidation conditions. Significant degradation of the drug was found under acid hydrolysis and photolysis condition.

Anti- rosuvastatin isomer and unknown impurities were formed. The main advantage of this method is the short run time, which helps in rapidly determining degradation products. The disadvantage is that the procedure for performing forced degradation studies is poorly explained. Reference [22], drug was found to be highly degraded under acid hydrolysis.

Lactone and an unknown impurity was the major degradation products formed under this hydrolysis. The drug was found to be comparatively stable under basic and photolytic conditions. An unknown degradant formed in acid hydrolysis was identified using UPLC-MS/MS analysis with Q-TOF mass spectrometer. A major degradant formed during acid stress was identified as ROS methyl ester. Analysis was performed using ESI in positive mode.

Advantages of the present work include a significant decrease in the consumption of solvent and time for separation and the identification of unknown impurities by coupling with QTOF mass spectrometer. The disadvantage is that forced degradation under neutral hydrolysis is not performed.

Recommendation: Here, reference [22] can be considered better because, in this work, the identification of the unknown degradant generated is performed using UPLC-MS/MS coupled with a Q-TOF mass spectrometer. Also, this work has various merits, such as less consumption of solvent and less time for separation of components.

TABLE 3: FORCED DEGRADATION STUDIES USING LC-UV-MS AND UV

Sl. no.	Acid Hydrolysis	Alkaline Hydrolysis	Neutral Hydrolysis	Oxidation	Thermal Hydrolysis	Photolysis	Ref.
1	0.1M HCl, 80 ºC, 15 min	0.1M NaOH heat for 1 hr		10% H_2O_2 80 ºC, 15 min	80 ºC, 1 hr	UV lamp, 20 min	23
2	0.1 N HCl, 60 ºC, 30 minutes reflux	0.1N NaOH, 60ºC, 30 minutes reflux		3% H_2O_2, 60 ºC, 30 minutes	60ºC,1 hour	UV light, 3 hours	24

Reference [23] shows the drug was highly unstable under acidic and photolysis under UV light. It is slightly sensitive to oxidation and heat. In total, eleven degradation products were formed. It was found to be relatively stable under basic conditions. Merit of this work is that the quantitative determination of degradation products was done using highly sensitive ESI – MS method. Information about degradation kinetics of rosuvastatin under UV exposure was explained. The proposed chemical structure of the degradation products was characterized by studying their MS^2 fragmentation pattern using Ion-trap MS in its acid form. The drug showed a molecular ion peak at m/z 482. Characteristic degradation product generated during oxidative stress was found to be Rosuvastatin –N-oxide

Reference [24] shows the drug's major degradation occurred under acid hydrolysis (15.64%). The degree of degradation of rosuvastatin is less when compared with that of metformin under various stress conditions. This method is useful for the quality control analysis of Rosuvastatin and Metformin in bulk and combined dosage forms. The disadvantage of the present work is that the number of degradation products formed under various stress conditions is not specified.

TABLE 4: FORCED DEGRADATION STUDIES USING TLC

Sl. no.	Acid Hydrolysis	Alkaline Hydrolysis	Neutral Hydrolysis	Oxidation	Thermal Hydrolysis	Photolysis	Ref.
1	0.1M HCl, 100 ºC, 5 min	0.1M NaOH 100ºC, 5 min		10% H_2O_2, 100ºC, 10 min	100ºC, 10 min	UV light (254nm)	25
2	1N HCl, 100ºC reflux, 3 hours						26

Reference [25], the drug was found to be highly susceptible to degradation under oxidation conditions, and five major degradation products were formed. Next significant degradation is seen under acidic hydrolysis, and four major degradants are formed. Two major degradation products are formed in the case of thermal degradation and photolysis. The mass spectroscopic analysis provided molecular ion peaks of different degradation products. Some of these degraded products were studied through their MS^2-MS^4 fragmentation pathway, while other unknown peaks cannot be interpreted. The chemical structures of four degradation products were characterized using LC-ESI-MS. The merit of this work is that the identification of the degradation products formed under various stress conditions is well explained. The degradation kinetics of the drug under UV irradiation is also explained. Demerit is that the forced degradation studies under neutral hydrolysis are not performed. Reference [26], drug was found to be sensitive under acidic hydrolysis and oxidation conditions. Two degradation products are formed when the drug is refluxed under acidic conditions. The drug is comparatively stable under alkaline hydrolysis. The structure of the degradation products generated during acid hydrolysis was determined through FT-IR and Mass spectroscopy. The disadvantage of this work is that the degradation of the drug under other stress conditions is not explained.

Recommendation: Here, reference [25] can be considered better and more useful in the future because, in this work, the characterization of degradation products generated during various stress conditions is performed using LC-ESI-MS, whereas in reference [26], only the characterization of the degradants generated during acid hydrolysis is explained and degradation of the drug under various conditions is not explained.

Forced Degradation Study of Teneligliptin by Using Various Analytical Techniques: The

optimum conditions for performing forced degradation studies are summarised in: **Table 5, Table 6, Table 7, Table 8, Table 9** and **Table 10.**

TABLE 5: FORCED DEGRADATION STUDIES USING RP- HPLC

Sl. no.	Acid Hydrolysis	Alkaline Hydrolysis	Neutral Hyrolysis	Oxidation	Thermal Hyrolysis	Photolysis	Ref
1	0.1 N HCl, 35 ºC,48 hrs	0.1N NaOH 35ºC, 48 hrs		3% H_2O_2, 35 ºC,48 hrs	69ºC, 48 hrs, reflux	UV,365nm 48 hrs	27
2	1N HCl, 60 ºC, 3 hrs	1 N NaOH, 60ºC, 3 hrs		3% H_2O_2, 60ºC, 3 hrs	105ºC, 6 hrs	UV,254nm, 24 hrs	28
3	2 N HCl, 60 ºC, 30 min	2N NaOH, 60ºC, 30 min	Water, 6hrs, 60ºC	20% H_2O_2, 60ºC, 30 min	105ºC, 6 hrs	UV, 7 days or 200Wh/m^2	29
4	1 N HCl, 1 hr, 50 ºC	1 N NaOH, 1 hr, 50 ºC		3% H_2O_2, 50ºC, 1 hr	105ºC, 5 hrs	Sunlight 5 days, RT	30
5	2N HCl, 60 ºC, 30 min	2 N NaOH, 60ºC, 30 min	Water, 6hrs, 60ºC	20% H_2O_2, 60ºC, 30 min	105ºC, 6 hrs	UV light- 7 days or 200Wh/m^2	31
6	0.1N HCl, 40 ºC, 4 hrs	0.1N NaOH RT, 4 hrs		3% H_2O_2, RT,4 hrs, 30min reflux	60ºC, 1 hr		32
7	0.1 N HCl, 8 hrs reflux	0.1N NaOH 8 hrs reflux	Water, 8 hrs reflux	3% H_2O_2	50ºC, 3 months	UV light – 15 days	33
8	0.5 N HCl, 2 hrs, 60 ºC	1 N NaOH, 5 hrs, RT		0.3 % H_2O_2, 12 hrs	80ºC, 2 days	1.2million lux hrs at 200Wh/m^2 near UV	34
9	1 M HCl, 50 ºC, 5 hrs	0.05 M NaOH, 50 ºC, 5 hrs	Double distilled water, 50 ºC, 5 hrs	10% H_2O_2, 50ºC, 5 hrs	50ºC, 48 hrs	UV light, 1 month	35

Discussion and Interpretation: For the degradation study done by authors of reference [27], degradation products formed are similar in the case of basic and thermal hydrolysis. Significant degradation of the drug was observed under oxidation, basic and thermal hydrolysis. Drug was found to be stable under acidic and photolysis condition. The degraded products were identified and characterized using UPLC with tandem mass spectroscopy (LC/MS/MS). The products were ionised by ESI mode for their mass data. The molecular ion peak for Teneligliptin was observed at 427.22. From the mass spectral data, the fragmentation pattern of Teneligliptin was portrayed. Fragmentation pattern of base, peroxide and thermally stressed drug was depicted. Disadvantage of this work is that the degradation of the drug under neutral conditions is not performed.

For reference [28], major degradation of the drug was observed under alkaline (19.5%) and acidic (15.33%) hydrolysis. The drug was found to follow first-order kinetics under alkaline hydrolysis. It indicates that as the temperature increases, the degradation of teneligliptin also increases. The merit of this work is that the degradation kinetics of the drug under alkaline hydrolysis is well explained. Demerit is that the number of degradation products formed under various stress conditions is not specified.

Reference [29], the drug was found to be significantly degraded under acidic hydrolysis (3.66%) and three degradation peaks were obtained. Two degradant peaks were found under alkaline hydrolysis and one peak under oxidation conditions. This method will be very useful for studying the degradation characteristics of Teneligliptin and Metformin in a combined dosage form.

Reference [30], major degradation of the drug has been observed under thermal hydrolysis (7.23%). Disadvantage of this work is that degradation of the drug under neutral conditions is not performed. Reference [31], significant degradation of the drug was found under acid hydrolysis (4.98%). For performing the dry heat and photolytic degradation, the sample was presented in the solid state. Degradation conditions such as the concentration of degradation reagent and time of exposure were optimized, so the degradation is within the range of 10%. The main demerit associated with this work is the long duration of the photolytic degradation (7 days).

Reference [32], the maximum degradation of the drug was found under oxidative conditions *i.e* 9.26%. The peak of the degradant does not interfere with the API peak. The merit of this work is that the degraded product can be well separated, quantified, and characterized so that its safety profile can be studied. Demerit is that the degradation of the drug under photolysis and the neutral condition is not performed.

Reference [33], the long duration of photolysis (15 days) and thermal degradation (3 months) is the main disadvantage of this work. Also, the degradation characteristics of the drug under various stress conditions are not explained.

Reference [34], the drug was significantly degraded under oxidative stress (11.6%). There is no interference of the degradant peak at the retention time of the main analyte peak. The demerit of this work is that neutral hydrolysis of the drug is not performed

Reference [35], the drug was found to be highly unstable under oxidation conditions (47%). It was found to be sensitive to acidic, alkaline, neutral, thermal, and photolytic degradation. The main disadvantage of this work is the long duration of photolytic degradation (1 month)

Recommendation: While going through all the forced degradation studies done using the RP-HPLC technique, it is seen that reference [27] is better when compared with other references. Because only in that work the degradation studies, identification, and characterization of degradation products are well explained. While comparing the references in which teneligliptin has undergone neutral hydrolysis, it is shown that reference [29] is better when compared with 33 and 35, because of the short duration of degradation studies and the number of degradant peaks are clearly mentioned here, when compared with other works.

TABLE 6: FORCED DEGRADATION STUDIES USING UHPLC

Sl. no.	Acid Hydrolysis	Alkaline Hydrolysis	Neutral Hydrolysis	Oxidation	Thermal Hydrolysis	Photolysis	Ref.
1	1 N HCl, 60ºC, 2 hrs	1N NaOH, 60ºC, 2 hrs		3%H_2O_2, 60ºC, 2 hrs	60ºC, 48 hrs	UV light (320–400 nm) 25ºC, 48hr	[36]

Reference [36], major degradation of the drug was found under oxidation conditions (27.59 %). It was found to be stable under thermal, photolysis, and acidic hydrolysis. This is a high speed, high-resolution analytical technique for simultaneous determination of Teneligliptin and Metformin in fixed dose combination. Demerit of this work is that, degradation under neutral conditions is not performed.

TABLE 7: FORCED DEGRADATION STUDIES USING UPLC

Sl. no.	Acid Hydrolysis	Alkaline Hydrolysis	Neutral Hydrolysis	Oxidation	Thermal Hydrolysis	Photolysis	Ref.
1	0.1 N HCl, 35ºC, 48hr	0.1N NaOH 35ºC, 48hr		3%H_2O_2, 35ºC, 48 hrs	100ºC, 48 hrs	UV light, 365nm, 48 hrs	[37]

Reference [37], significant degradation of the drug was observed under oxidation and alkaline hydrolysis. Degradation products were found to be toxic when analyzed using Osiris software. The drug was found to be stable under thermal, photolysis and acidic hydrolysis. The degradation products were characterized using IR, NMR and MS techniques to get detailed information about their structure. H1 and C13 NMR were also recorded. The demerit of this work is that the degradation under neutral conditions is not performed.

TABLE 8: FORCED DEGRADATION STUDIES USING RP- UFLC

Sl. no.	Acid Hydrolysis	Alkaline Hydrolysis	Neutral Hydrolysis	Oxidation	Thermal Hydrolysis	Photolysis	Ref.
1	0.1 M HCl	0.1M NaOH		3% H_2O_2	60 ºC	UV lamp, 45 ºC	[38]
2	0.1 N HCl, 60 ºC, 30 min	0.1N NaOH 60 ºC, 30 min	Water,60 ºC	30% H_2O_2 60ºC, 30min			[39]

Reference [38], the percentage recovery of the drug was found to be very less after oxidation conditions (22.23%). A disadvantage of this work is that degradation under neutral conditions is not performed as well as the number of degradation products formed under various stress conditions is not specified. Reference [39], peak of Teneligliptin was completely destroyed under acidic and alkaline hydrolysis due to carbonyl moiety and heterocyclic moiety in the structure of the drug. Less than 5% degradation was observed under oxidation and neutral hydrolysis. The disadvantage of this work is that degradation under photolysis and thermal condition is not performed.

TABLE 9: FORCED DEGRADATION STUDIES USING UV

Sl. no.	Acid Hydrolysis	Alkaline Hydrolysis	Neutral Hydrolysis	Oxidation	Thermal Hydrolysis	Photolysis	Ref.
1	0.1 N HCl, 60ºC, 30 min reflux	0.1M NaOH 60ºC, 30 min reflux		30% H_2O_2, 60ºC, 30 min reflux	80ºC, 6 hrs	Sunlight, 30 min	[40]
2	0.1 N HCl, 60ºC, 4 hr	0.1N NaOH 60ºC, 4hrs		3% H_2O_2, 60ºC, 4hrs	60ºC, 4 hrs	UV – 4 hrs Sunlight – 24 hrs	[41]

Reference [40] found that when the amount of stress applied increases, the degradation rate also increases. Major degradation of the drug occurred under photolysis conditions using UV light. The area of the degraded peaks was less than the area of the standard drug peak; this indicates that the drug undergoes degradation in all conditions. The demerit of this work is that the degradation under neutral conditions is not performed. Reference [41], major degradation of the drug was found under oxidation and alkaline hydrolysis. In sunlight degradation, both Teneligliptin and Metformin show the lowest degradation. The disadvantage of this work is that the number of degradation products formed under various stress conditions is not explained.

Recommendation: While going through the forced degradation studies done using UV spectroscopy, it is shown that reference [40] is better when compared with [41] because of the short duration of degradation studies. Also, this reference explains the procedure for performing degradation studies under various stress conditions.

TABLE 10: FORCED DEGRADATION STUDIES USING HPTLC

Sl. no.	Acid Hydrolysis	Alkaline Hydrolysis	Neutral Hydrolysis	Oxidation	Thermal Hydrolysis	Photolysis	Ref.
1	2 M HCl, 80ºC, 3 hrs	0.05M NaOH, 80ºC3 hrs	Distilled water, 80 ºC, 3 hrs	3% H_2O_2, 80ºC, 3 hrs	60ºC, 24 hrs	UV light – 254 nm, 24 hrs	[42]

Reference [42], significant degradation of the drug was found under alkaline hydrolysis and oxidation stress conditions. This method can be applied for the routine quality control analysis of the drug in bulk and pharmaceutical dosage form. A disadvantage of this work is the long duration of photolysis and thermal hydrolysis (24 hrs) when compared with other stress conditions.

CONCLUSION: With the help of forced degradation studies, we can establish degradation pathways of the drug substance. It will also be very useful to understand the conditions in which the drug undergoes significant degradation. This information is vital for selecting proper formulation, packaging and the determination of shelf life. The above article explains the forced degradation studies conducted on rosuvastatin and teneligliptin using various analytical techniques. By going through this information, readers will understand the nature of the degradation of both drugs.

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