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DEVELOPMENT OF STABILITY INDICATING HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHIC METHOD FOR SIMULTANEOUS ESTIMATION OF TENELI-GLIPTIN AND METFORMIN: APPLICATION TO ALKALINE DEGRADATION KINETIC STUDY

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Keywords:

Stability indicating HPTLC method, Degradation kinetic study, Degradation products, LC-Mass spectroscopy

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ABSTRACT: Stability indicating the HPTLC method was developed for simultaneous estimation of teneligliptin and metformin by using aluminum plates precoated with silica gel 60 F224 (Merck) and mobile phase toluene: methanol: GAA: TEA (5:4:0.5:0.5) with scanning wavelength of 257 nm. The developed method was validated for linearity, stability, accuracy, precision, robustness, LOD and LOQ as per ICH guidelines. The proposed method was successfully applied for the assay of metformin and teneligliptin in dosage forms and results were found to be in an acceptable range with a labeled claim of the pharmaceutical dosage form. Linearity for metformin and teneligliptin was observed\with correlation coefficient (r^2) value of 0.999 and 0.993. The proposed method was also applied for degradation study of dosage form in alkaline acidic, Thermal, and photolytic conditions. Further, kinetic degradation study in alkaline conditions (0.1 N, 0.5 N, 1.0 N NaOH) at 60 °C and 80 °C are carried out. The degradation rate constant was also predicted at different temperatures and strengths of NaOH using kinetic methods. Degraded product of metformin and teneligliptin formed in the alkaline medium were isolated and characterized using Mass spectroscopy.

INTRODUCTION: Teneligliptin hydrobromide hydrate (TENE) is chemically described as {(2 S, 4 S) - 4 - [4 - (3 - methyl - 1 phenyl-1H-pyrazol-5-yl) piperazin-1-yl] pyrrolidin-2-yl} (1, 3-thiazolidin-3-yl) methadone hemipenta hydrobromide hydrate is a dipeptidyl peptidase inhibitor **Fig. 1**. TENE shows reducing fasting and postprandial glucose concentrations in a glucose-dependant manner in patients with type 2 diabetes mellitus.

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Metformin hydrochloride (MET) is 1, 1-dimethyl biguanide hydrochloride, a biguanide ant diabetic **Fig. 2**. It is given orally in the treatment of type 2 diabetes mellitus and is the drug of choice in overweight patients. For effective control of blood sugar in diabetic patients, more than one medication is required. TENE shows effective control of blood sugar when combined with MET.

Determination of the intrinsic stability of the drug and its degradation products for the development of pharmaceutical formulation is very important. Degradation study allows giving an idea about its therapeutic and physiochemical properties. The stability of the drug should be evaluated in terms of temperature, humidity, oxidation, UV light exposure, and hydrolysis.

Degradation kinetic study helps to develop optimum formulation in preformulation studies with the optimum storage conditions (temperature, light, humidity). It also helps to predict the shelf life of the drug and anticipating drug excipient interactions. A literature survey reveals various HPLC and UV methods for the determination of MET and TENE in bulk and dosage forms, some stability-indicating HPLC studies for MET and TENE were also reported [2-6]. Literature survey also revealed many methods for estimation of TENE in a dosage form; some also reveals the methods for estimation of MET with other drugs combination [7-13]. Very few HPTLC methods are reported for this combination. A literature study was also not found revealing the degradation kinetic data for TENE and MET in alkaline medium. HPTLC is used for routine analysis because its advantages like many samples can be run at a time using a small volume of mobile phases unlike HPLC and nearby lowers the cost and time of the analysis. Mobile phase having higher pH can also be used for the analysis. HPTLC is also used for the simultaneous estimation of multicomponent in a formulation. The objective of the present study was to develop a novel, simple, accurate, precise, economic stability-indicating assay method for the simultaneous estimation of metformin hydrochloride and teneligliptin and to study alkaline degradation kinetics validate the method with forced degradation studies according to ICH guidelines [1].

EXPERIMENTAL:

Stability Indicating Assay Method for TENE and MET High-Performance Thin Layer Chromatography: The HPTLC system (Make: camag) with sample applicator: camag linomat V (Muttenz, Switzerland), UV-lamp, camag TLC scanner III densitometer operated in reflectance-absorbance mode and syringe: camag 100 µl syringe (Hamilton, Bonaduz, Switzerland), TLC plates used are aluminum plates percolated with silica gel 60 F 254 plates (E. Merck, Darmstadt, Germany; supplied by Merck India, Mumbai, India) with camag twin trough glass development chamber.

Chemical and Reagent: Metformin hydrochloride and teneligliptin hydrobromide procured as a gift sample from spectrum Private Ltd. Hyderabad (The drug samples were used as such without further, purification). Marketed formulation tenglyn M 500 was procured from the local market.

Reagents and Solvents: Toluene, methanol, glacial acetic acid and triethylamine solvents of AR grade were used for analysis.

Chromatographic Conditions: Chromatographic conditions are optimized by trial and error for estimation of MET and TENE. The mobile phase selected for analysis was toluene: methanol: GAA: tea (5: 4: 0.5: 0.5) with aluminum plates precoated with silica gel 60 F 224 (Merck) as a stationary phase. TLC plates are prewashed with methanol and dried. The sample was applied with the help of sample applicator: camag linomat V. Twin-through glass chamber, 10 cm × 10 cm with a stainless steel lid was used as a development chamber. The TLC plate was scanned and analyzed by TLC scanner and win CATS software using; detection wavelength, 257 nm.

Preparation of Working Standard Solution of THC: Standard solutions of MET and TENE were prepared by dissolving 250 mg of MET and 10 mg of TENE in 10 ml volumetric flask, volume was made up to the mark with methanol to obtain the final concentration of 25 mg/ml and 1 mg/ml respectively.

Sample Preparation for Forced Degradation Studies: For forced degradation studies, the drugs were subjected to acidic, alkaline, oxidative, thermal and photolytic degradation

Acidic Degradation: 0.1 N HCL was added to a 10 ml volumetric flask containing a mixture of 250 mg of MET and 10 mg of TENE. The flask was kept on a water bath at 80 °C for 2 h and further, dilute with methanol and developed the plate using optimized chromatographic conditions.

Alkaline Degradation: 0.5 N NaOH was added to a 10 ml volumetric flask containing a mixture of 250 mg of MET and 10 mg of TENE. The flask kept on a water bath at 60 °C for 2 h and further, dilute with methanol and developed the plate using optimized chromatographic conditions.

Oxidative Degradation: 3% Hydrogen peroxide was added to a 10 ml volumetric flask containing a mixture of 250 mg of MET and 10 mg of TENE.

The flask kept on a water bath at 60 °C for 2 h and further diluted up to methanol and the sample solutions were applied (12.52 µg /band of MET and 500 ng/band of TENE) and developed the plate using optimized chromatographic condition.

Thermal Degradation: For dry heat degradation, the standard drugs were placed in a hot air oven at 80 °C for 60 min. Appropriate dilutions were made using methanol and analyzed under the optimized chromatographic conditions.

Photo Stability Studies: For photostability study, the standard drugs were exposed to UV light in the photostability chamber for 24 h at 254 nm. Appropriate dilutions were made using methanol and analyzed under the optimized chromatographic conditions. The MET and TENE were stable when subjected to photolytic degradation.

Procedure for Calibration Curve: The working standard solution of MET and TENE was applied on the TLC plates in the range 0.3 to 0.8 µl *i.e.* 7.5 -20.00 µg/band of MET and 300-800 ng/band of TENE with the help of hamilton syringe using linomat-V automatic sample applicator.

The plate was then developed in optimized mobile phase. Densitometry evaluations of the drugs were performed at 257 nm. Peak area was recorded. The peak areas were plotted against the corresponding concentration to obtain the calibration graph.

Analytical Method Validation: Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications.

Accuracy: To ascertain the accuracy of the proposed methods, recovery studies were carried out by standard addition method at 80, 100 and 120 % of the test concentration as per ICH guidelines. The amount of the drug recovered (mg) and percent recovery was calculated.

Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Precision may be considered at three levels: repeatability, intermediate precision, and reproducibility.

Repeatability: Six samples of the preparation of the marketed tablet were analyzed as per the procedure given under the analysis of the same. The standard deviation (S. D.), % Relative standard deviation (%RSD) was calculated.

Intermediate Precision (Intra-day and Inter-Day Precision): Intraday and interlay precision was determined by analyzing tablet sample solutions at different time intervals on the same day and on three different days, respectively.

Detection Limit: The LOD and LOQ were separately determined which is based on the standard deviation of the response of the calibration curve. The standard deviation of the y-intercept and slope of the calibration curves were used to calculate the LOD and LOQ.

Linearity: Linear relation was found in the range of 12.50 -20.00 µg, band and 300-800 ng/band with a correlation of coefficient (r^2) of 0.999 and 0.993 for MET and TENE respectively.

Robustness: To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters such as the composition of the mobile phase, chamber saturation time, time from spotting to development, development to scanning, the volume of the mobile phase, and stability of the solution, were done. The composition of the mobile phase and chamber saturation time were varied in the range of ± 0.1 ml and ± 2 min, respectively, of the used optimized conditions. The volume of mobile phase was varied by ± 1 ml. The effect of these changes on both the R_f values and peak area was studied.

Procedure for Assay of Marketed Formulations:
Preparation of Standard Stock Solution: Twenty tablets (Tenglyn M) were weighed and crushed to obtain a fine powder.

The average weight of the tablets was calculated. Accurately weighed quantity of tablet powder equivalent to about 250 mg of MET and 10 mg of TENE was transferred to 10.0 ml volumetric flask, added few ml of methanol and ultrasonicated for 10

min, volume was then made up to the mark with methanol. On the TLC plate bands of standard stock solution and bands of the sample solution, 0.5 µl each, was applied and the plate was developed and scanned under the optimum chromatographic condition. After scanning, the peak obtained for standard and sample bands were integrated.

Preparation of Sample for Alkaline Kinetic Degradation Study: Different strengths (0.1, 0.5 and 1.0 N) of sodium hydroxide solutions were prepared and transferred in three different 10 ml volumetric flasks. Weigh the quantity of 250 mg MET and 10 mg of TENE in each separate 10 ml volumetric flask. Add 3 ml of each concentration (0.1, 0.5, 1.0, N) of NaOH to a volumetric flask containing the weighed quantity of MET and TENE.

The volumetric flasks were placed in a controlled temperature water bath at 60 and 80 °C separately for 3 h. Samples were taken out at an interval of 1 h up to 3 h and make up the volume up to the mark with methanol. The sample was applied over the TLC plate and analyzed using the optimized chromatographic condition. A graph was plotted of percent log remaining *vs*. time. From the data of degradation kinetic study degradation rate constant, half-life and shelf life for THC were calculated.

Isolation of Degradation Product by using HPTLC Method: Accurately weighed quantity of 250 mg of MET and 10 mg of TENE were separately transferred to different 10.0 ml volumetric flasks, added 3.0 ml of 1 N HCL and 1 N NaOH respectively. The forced degradation study was carried out by exposing samples to the above stress conditions. The contents of the flask were reflux in a water bath at 80 °C for about 3 h. After the respective time intervals, all the flasks were removed and allowed to cool and diluted up to mark with methanol. Then the samples were applied on the TLC plate with the sample volume of about 10 µl/band, 6 bands of the degraded samples were applied. Then the TLC plates were placed in a developing chamber using optimized chromate-graphic conditions.

After the development of the plate, these plates were observed under the UV chamber and band of the std. and degradation product was marked and further scraped and extracted with methanol. The sample was sent for LC-MS studies for interpretation of the probable structure of the degradation product.

RESULTS AND DISCUSSION:

Selection of Mobile Phase: Appropriate dilutions of the stock solutions were prepared and applied on TLC plates in the form of the band (band size: 6 mm) and the plates were run in different solvent systems. Different mobile phase systems consisting of different ratios of toluene, methanol, chloroform, GAA were tried in order to determine the best condition for the effective separation of MET and TENE. Among the different mobile phase combinations tested toluene: methanol: GAA: TEA (5: 4: 0.5: 0.5) was selected as it gives good resolution and peak symmetry for MET and TENE. The R_f value for MET and TENE was found to be 0.56 and 0.75 respectively **Fig. 1.**

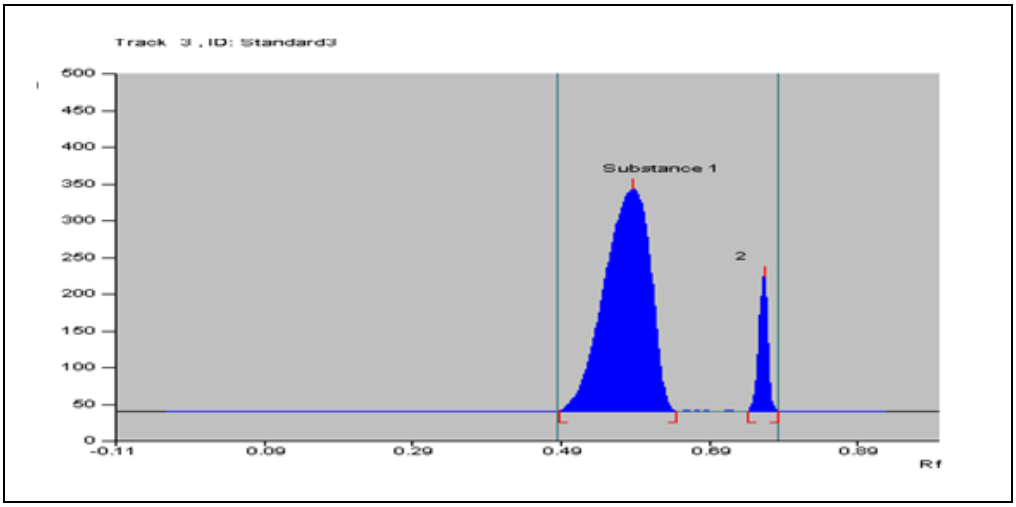

FIG. 1: TYPICAL DENSITIOGRAM OF MET AND TENE

Selection of Analytical Wavelength for Densitometric Evaluation: Standard stock solution was applied on the TLC plate with the help of camag linomat-v automatic sample applicator, in order to determine the absorbance maxima. The plate was developed in a twin-through glass chamber saturated with mobile phase for 10 min.

After chromatographic development, the plate was removed and air-dried. The bands on the TLC plate were scanned over the wavelength range of 200700 nm. Both drugs showed absorbance and good resolution at 257 nm which is selected as an analytical wavelength.

Optimized Chromatographic Conditions: The following chromatographic conditions were optimized by trial and error for densitometric analysis of MET and TENE as shown in **Table 1.**

TABLE 1: OPTIMISED CHROMATOGRAPHIC CONDITIONS

Stationary phase	Aluminum plates percolated with silica gel 60 F_{224} (Merck)
Mobile phase	Toluene: Methanol: GAA: TEA (5: 4: 0.5: 0.5)
Plate size	10 cm X 10 cm
Mode of application	Band
Band size	6 mm (Distance between two bands: 5.6 mm) 0.5µl
Sample application volume	
Development chamber	Twin-through glass chamber, 10 cm × 10 cm with stainless steel lid
Saturation time	10 min

Forced Degradation Studies: For forced degradation studies, the drugs were subjected to acidic, alkaline, oxidative, thermal and photolytic degradation.

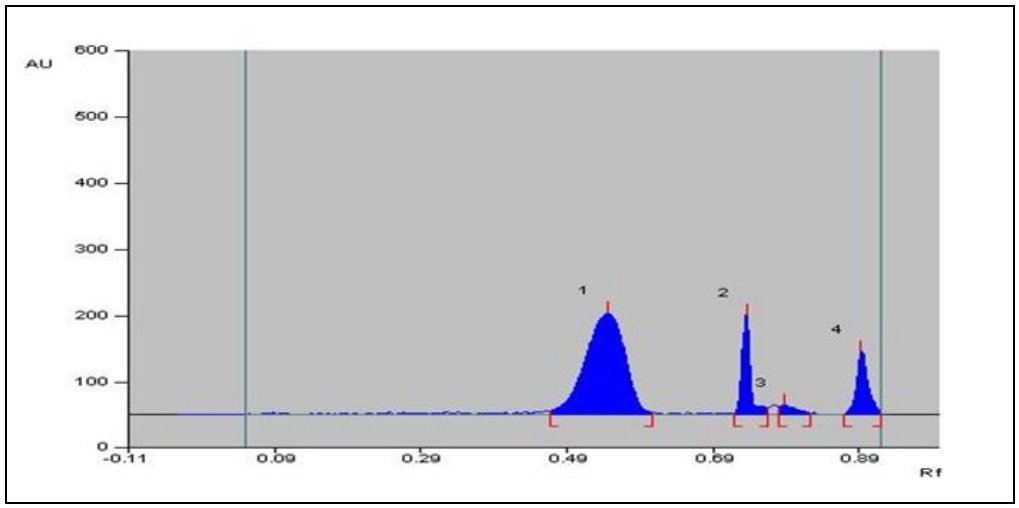

FIG. 2: DENSITOGRAM OF ACID (0.1 N HCL) TREATED MET AND TENE (DEGRADATION PEAK RECORDED AT 1] 0.87 2] 0.89)

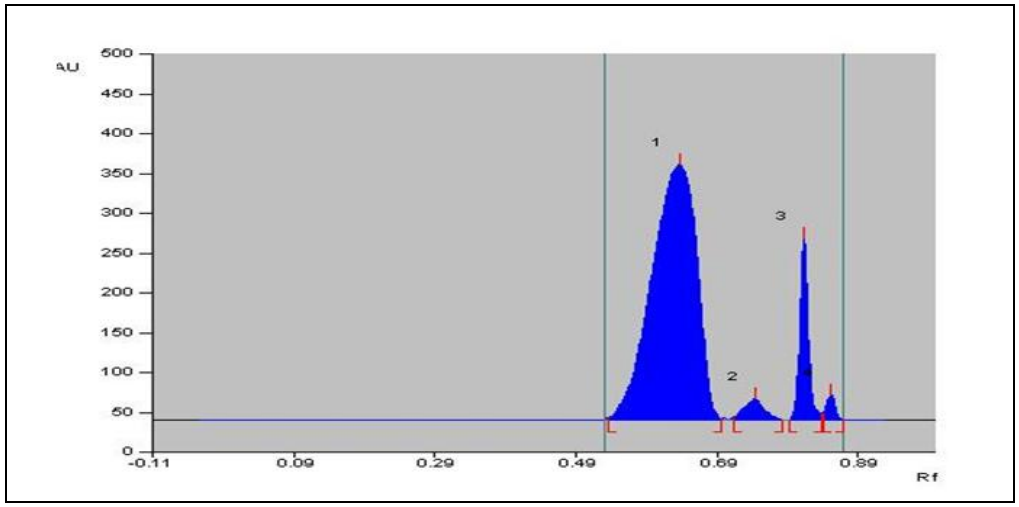

FIG. 3: DENSITOGRAM OF ALKALINE (0.5 N NAOH) TREATED MET AND TENE (DEGRADATION PEAK RECORDED AT 1] 0.69 2] 0.87 3] 0.89

In the acidic degradation study, the MET was degraded 19.72% and TENE up to 23.73% and degradation products were observed at R_f of 0.87 and 0.89. **Fig. 2** and **Table 2**. In alkaline conditions, the MET was degraded 16.47% and TENE up to 7.97% and degradation products were observed at Rf of 0.69 0.87 and 0.89. **Fig. 3** and **Table 2**. On exposure to 3% hydrogen peroxide, the MET was degraded 12.88% and TENE up to 27.94 % and degradation products were observed at R_f of 0.63 and 0.68. **Fig. 4** and **Table 2**.

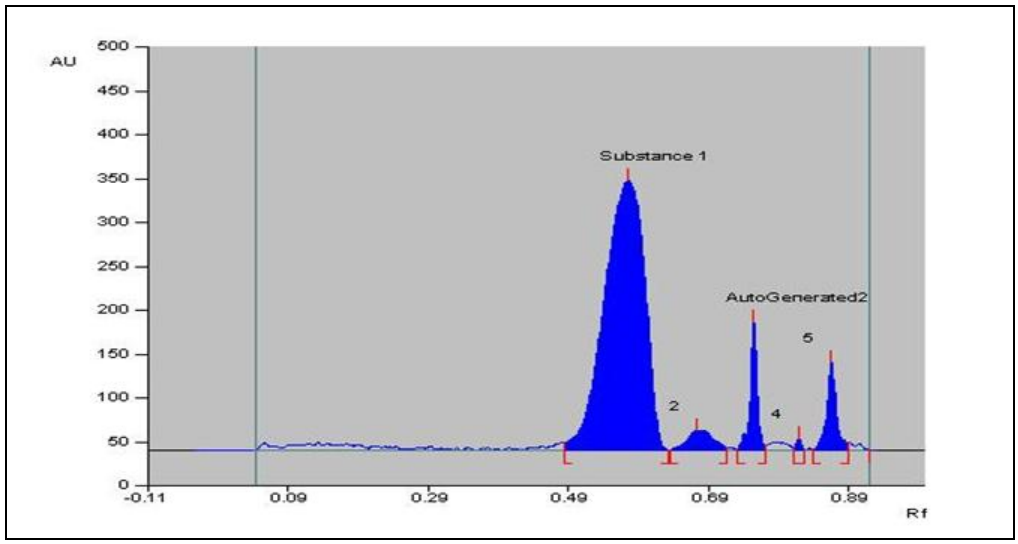

FIG. 4: DENSITOGRAM OF OXIDATIVE (3% H_2O_2) TREATED MET AND TENE (DEGRADATION PEAK RECORDED AT 1] 0.63 2] 0.68)

On dry heat degradation study, the MET was degraded 9.01% and TENE up to 8.77% and the degradation product was observed at R_f of 0.74. **Fig. 5** and **Table 2**. On exposure to UV light in the photostability chamber for 24 h at 254 nm, both the drugs were found to be stable.

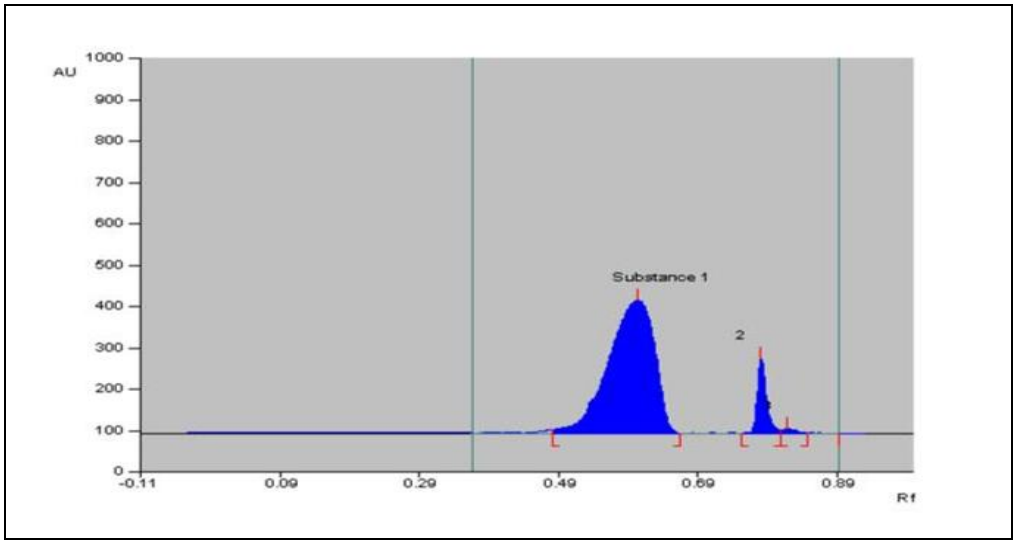

FIG. 5: DENSITOGRAM OF THERMAL TREATED MET AND TENE

TABLE 2: RESULT OF DEGRADATION STUDIES

S. no.	Stress condition	Temperature and time	% assay of active substance		R_f of degraded product
			MET	TENE	
1	Acid (0.1 N HCl)	60 °C for 2 h	80.28	73.73	0.87, 0.89
2	Alkali (0.5 N NaOH)	60 °C for 2 h	83.53	92.03	0.69, 0.87, 0.89
3	Oxide (3 % H_2O_2)	60 °C for 2 h	87.12	72.06	0.63, 0.68
4	Thermal	80 °C for 1hr	90.99	91.23	0.74
5	Photo degradation	254 nm for 24 h	100.45	100.78	-

Method Validation:

Preparation of Calibration Curve: Calibration curve for MET and TENE was found in the range 0.3 to 0.8 µl *i.e.* 7.5-20.00 µg / band of MET and 300-800 ng/band of TENE. The standard calibration curve for MET and TENE is shown in **Fig. 6A** and **6B** and calibration data are shown in **Table 3**.

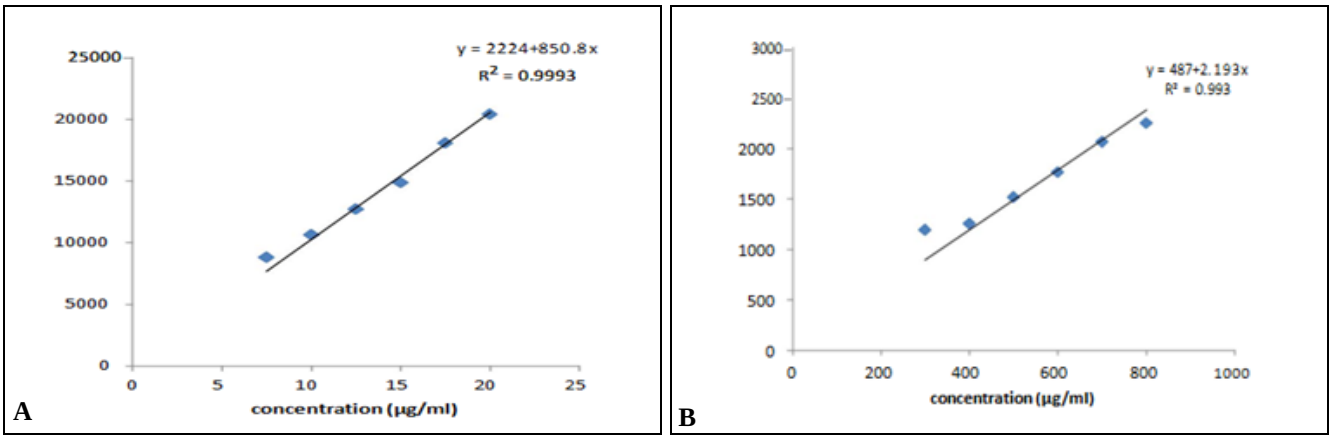

FIG. 6A: CALIBRATION CURVE FOR MET B CALIBRATION CURVE FOR TENE

TABLE 5: STANDARD CALIBRATION DATA OF MET/ TENE

S. no.	Concentration (µg/ml)	Peak area	Concentration (µg/ml)	Peak area
	Metformin		Teneligliptin	
1	7.50	8807.34	300	1195.06
2	10.00	10650.10	400	1356.72
3	12.50	12720.59	500	1518.94
4	15.00	14867.20	600	1765.70
5	17.50	17080.20	700	2067.92
6	20.00	19409.38	800	2253.92

Accuracy: To ascertain the accuracy of the proposed methods, recovery studies were carried out by standard addition method at 80, 100 and 120 % of the test concentration as per ICH guidelines. The amount of the drug recovered (mg) and percent recovery was calculated and results of recovery studies and its statistical validation are shown in **Table 6**.

TABLE 6: STATISTICAL VALIDATION FOR RECOVERY STUDY

Level	% Mean recovery		S. D.		% RSD	
	MET	TENE	MET	TENE	MET	TENE
80	100.56	100.19	1.50	0.600	1.49	0.598
100	101.12	100.24	0.973	0.582	0.962	0.580
120	99.44	99.73	0.4996	0.556	0.502	0.557

Precision:

Repeatability: To check the degree of repeatability of the method, suitable statistical evaluation was carried out. Six samples of the preparation of the marketed tablet were analyzed as per the procedure given under the analysis of the same. The standard deviation (S.D.), % relative standard deviation (%RSD) was calculated. The results are given in **Table 7**.

Intermediate Precision (Intra-Day and Inter-Day Precision): Intraday and inter-day precision were determined by analyzing tablet sample solutions at different time intervals on the same day and three different days, respectively. Results of intra-day and inter-day precision are shown in **Table 7**.

Limit of Detection and Limit of Quantification: The LOD and LOQ were separately determined which is based on the standard deviation of the response of the calibration curve. The standard deviation of the y-intercept and slope of the calibration curves was used to calculate the LOD and LOQ. Results of LOD and LOQ are shown in **Table 8**.

TABLE 7: RESULT OF INTRA-DAY AND INTER-DAY PRECISION OF HPTLC METHOD

Precision	% label claim		S. D.		% RSD	
	MET	TENE	MET	TENE	MET	TENE
Repeatability	99.67	101.91	0.166	0.625	0.167	0.628
Intraday precision	98.48	100.61	0.083	0.744	0.085	0.74
Interday precision	99.40	99.74	0.734	0.808	0.739	0.811

TABLE 8: RESULT OF LOD AND LOQ

S. no.	Components	LOD (µg/ml)	LOQ (µg/ml)
1	MET	0.0244	0.0267
2	TENE	0.07399	0.0811

Robustness: To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The composition of the mobile phase and chamber saturation time were varied in the range of ± 0.1 ml and ± 2 min, respectively, of the used optimized conditions. The volume of mobile phase was varied by ± 1 ml. The effect of these changes on both the R_f values and peak area was studied. Result of robustness studies and its statistical validation are shown in **Table 9**.

TABLE 9: RESULT OF ROBUSTNESS STUDY

Factor	% RSD	
	MET	TENE
Mobile phase composition (± 0.1 ml)	0.674	1.715
Duration for chamber saturation (± 2 min)	0.198	1.765
Volume of mobile phase (± 1 ml)	0.757	1.356

Analysis of Marketed Formulation: The proposed method was applied for assay the tablet dosage forms containing TENE and MET and % amount of both drugs were found to be 101.28% and 100.81% of a labeled claim of MET and TENE respectively. Results are shown in **Table 10**. Six samples were prepared and analyzed in following manner. The chromatogram is shown in **Fig. 7.**

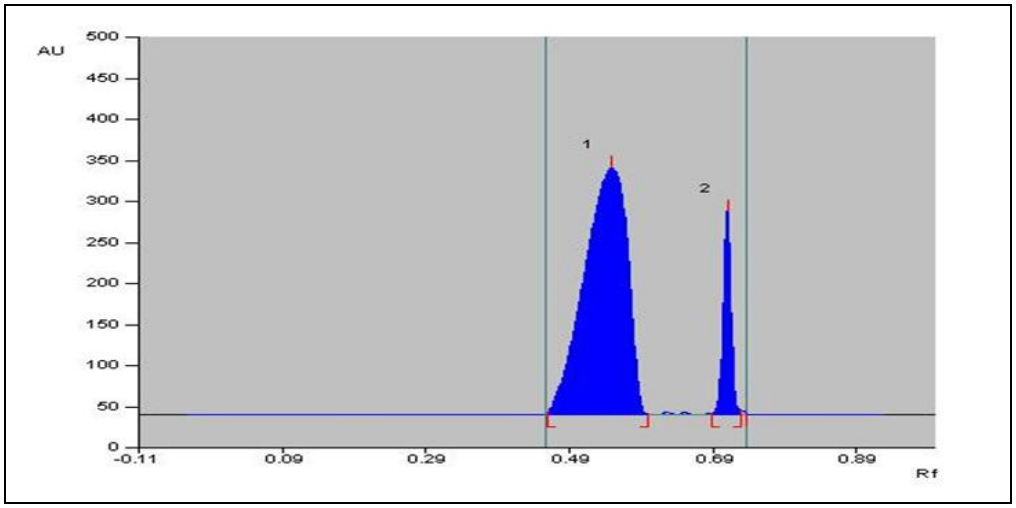

FIG. 7: TYPICAL DENSITOGRAM OF MARKETED FORMULATION

Alkaline Kinetic Degradation Study: The alkaline degradation kinetic study of MET and TENE was performed in 0.1, 0.5 and 1.0N NaOH solutions at 60 and 80 °C using developed HPTLC method for the estimation of MET and TENE in a dosage form.

The degradation kinetics was helpful for the determination of the shelf life, half-life and rate constant of the drug product. The degradation rate constant and half-life for MET and TENE were determined for each set of data in **Table 11**. The alkaline degradation of MET and TENE were found to follow the first-order kinetic and the highest degradation of MET and TENE was found in 1.0 N NaOH at 80 °C **Fig. 8A-8D**.

TABLE 10: RESULTS OF ANALYSIS OF MARKETED FORMULATION

Drugs	Amount of drug found	% Label claim	S. D.	% RSD
MET	506.099	101.28	1.08	1.10
TENE	20.19	100.81	1.76	1.78

Effect of Strength of Sodium Hydroxide on Degradation of MET and TENE: At different temperatures, as the strength of sodium hydroxide increases from 0.1 to 1.0 N, the degradation rate constant increases and the half-life decreases for

alkaline degradation of MET and TENE. **Fig. 9A-B** and **Fig. 10A-B**.

Effect of Temperature on Degradation of MET and TENE: At different strengths of sodium hydroxide, as the temperature increases from 60 to 80 °C, the degradation rate constant increases and the half-life decreases for alkaline degradation of MET and TENE.

The % degradation of MET and TENE and the degradation rate constant increase either temperature increases or strength of NaOH increases or both increase while the degradation half-life decreases either strength of NaOH increases or temperature increases or both increase Results of degradation kinetics are given below in **Table 11**.

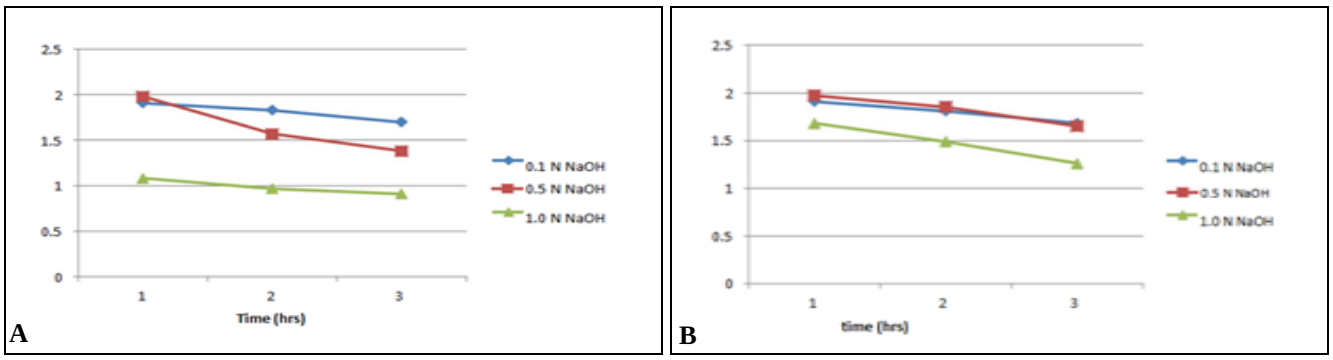

FIG. 8A-B: DEGRADATION DATA OF MET IN 0.1, 0.5 AND 1.0 N NaOH AT 80 °C

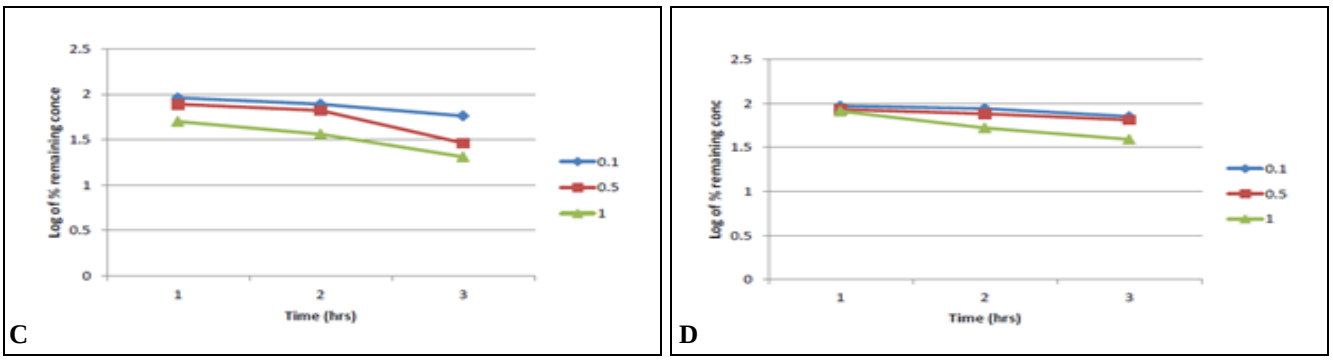

FIG. 8C-D: DEGRADATION DATA OF MET IN 0.1, 0.5 AND 1.0 N NaOH AT 60 °C

TABLE 11: SUMMARY OF ALKALINE KINETIC DEGRADATION STUDY

Temperature (ºC)	Strength of NaOH (N)	Degradation rate constant, K (per h)		Half life (hr)		Order of reaction	
		MET	TENE	MET	TENE	MET	TENE
60	0.1	0.1884	0.1381	3.67	5.018	First	First
	0.5	0.2072	0.1831	3.34	4.03	First	First
	1.0	0.633325	0.3684	1.09	1.8	First	First
80	0.1	0.24181	0.26484	2.86	2.61	First	First
	0.5	0.4490	0.3684	1.54	1.88	First	First
	1.0	0.6909	0.4836	1.003	1.44	First	First

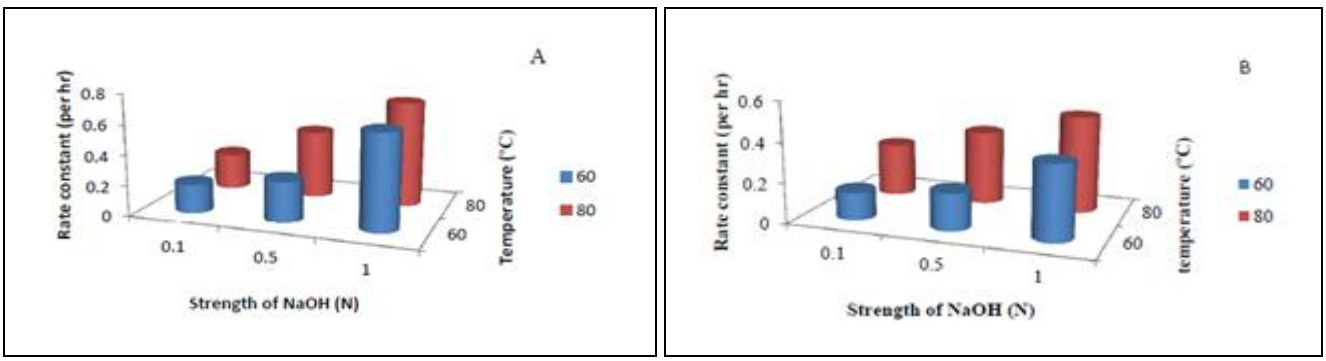

FIG. 9: COMPARISON OF DEGRADATION RATE OF (A) MET (B) TENE IN ALKALINE MEDIUM

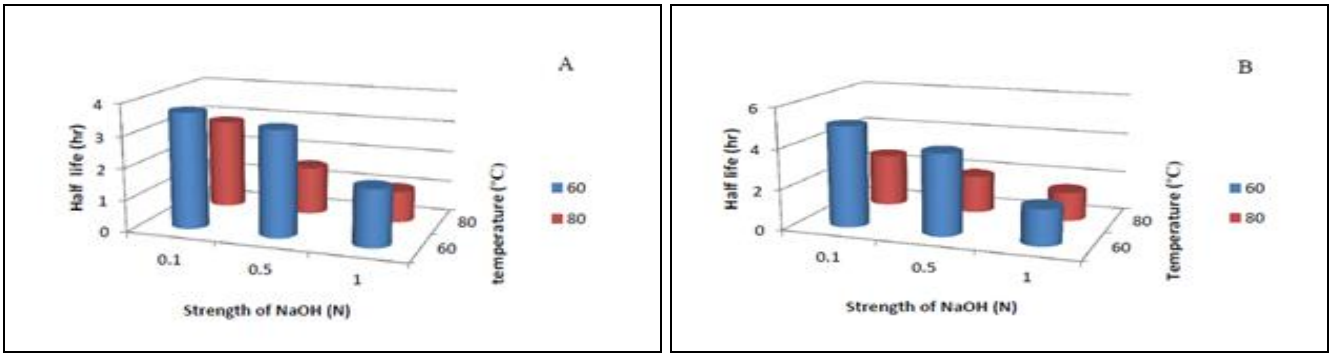

FIG. 10: COMPARISON OF HALF-LIFE OF (A) MET (B) TENE IN ALKALINE MEDIUM

Energy of Activation (Ea): A plot of in k as a function of 1 / T referred to as the Arrhenius plot is linear if Ea is independent of temperature. The slope of line obtained from the plot of ln k versus 1 / T is equal to - Ea / R. Results are shown in **Table 12**. - Ea / R = slope

TABLE 12: DATA FOR ENERGY OF ACTIVATION

Strength of NaOH (N)		0.1	0.5	1.0
Energy of activation (kcal /mol)	7.490	6.72	3.99	3.99
	6.044	5.57	4.19	4.19

Characterization of Degraded Products: In mass spectra, MET shows a molecular ion peak at 129.09 in acidic and alkaline stress degradation conditions.

The base peak for MET was observed at 101.19 in acidic and alkaline stress degradation conditions that indicate the molecular weight of degraded product DPI was found to be 101 amu and confirm the absence of two NH moiety (Mol. Wt = 30 amu) from metformin **Fig. 11** and **12**.

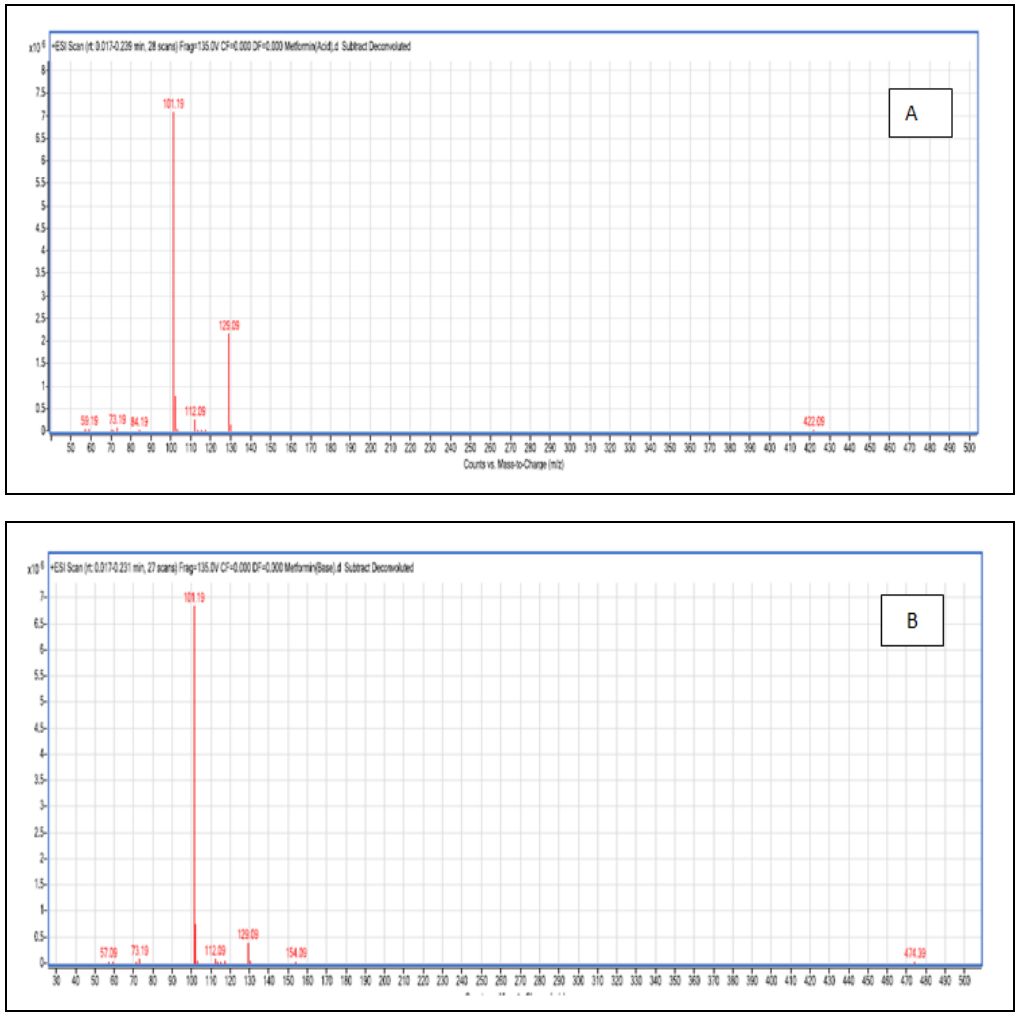

FIG. 11: MS SPECTRA OF MET AT (A) ACID STRESS (B) ALKALINE STRES

TENE shows molecular ion peak at 423.99 and 424.09 in acidic and alkaline stress degradation conditions. The base peak for TENE was observed at 205.09 and 147.99 in acidic and alkaline stress degradation conditions that indicate the molecular weight of degraded product DP4 and DP5 were found to be 199 amu and 152 amu respectively **Fig. 13** and **14**.

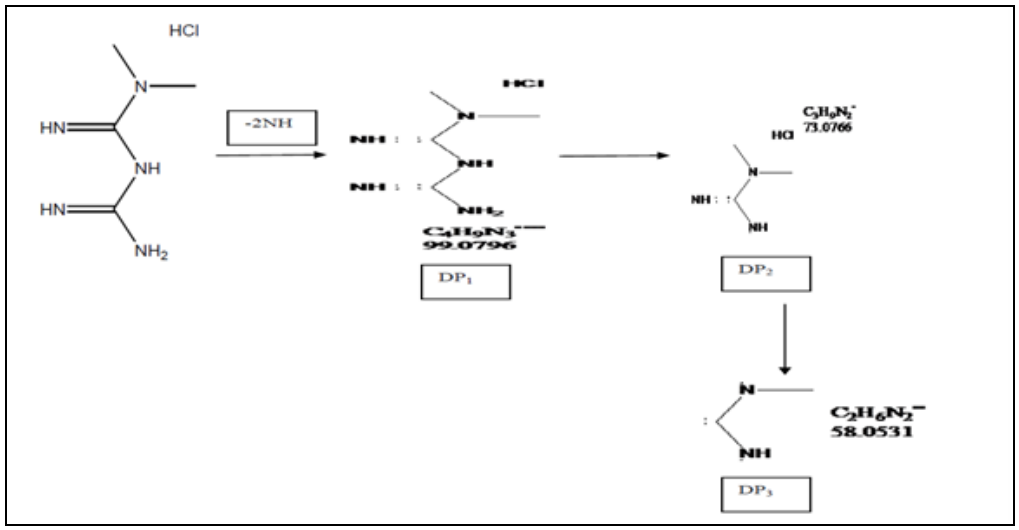

FIG. 12: DEGRADATION PATHWAY OF MET IN ACID AS WELL AS ALKALINE STRESS CONDITION

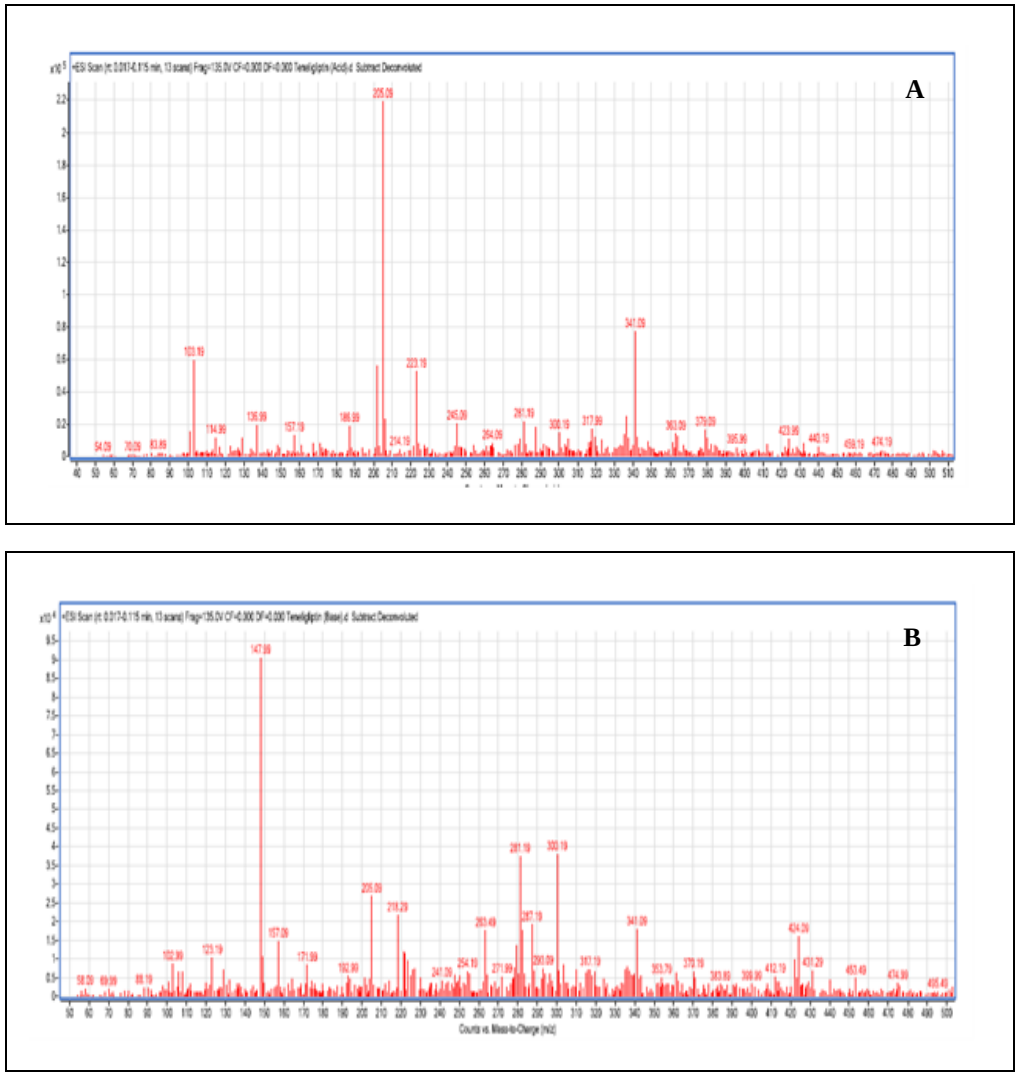

FIG. 13: MS SPECTRA OF TENE AT (A) ACID STRESS (B) ALKALINE STRESS

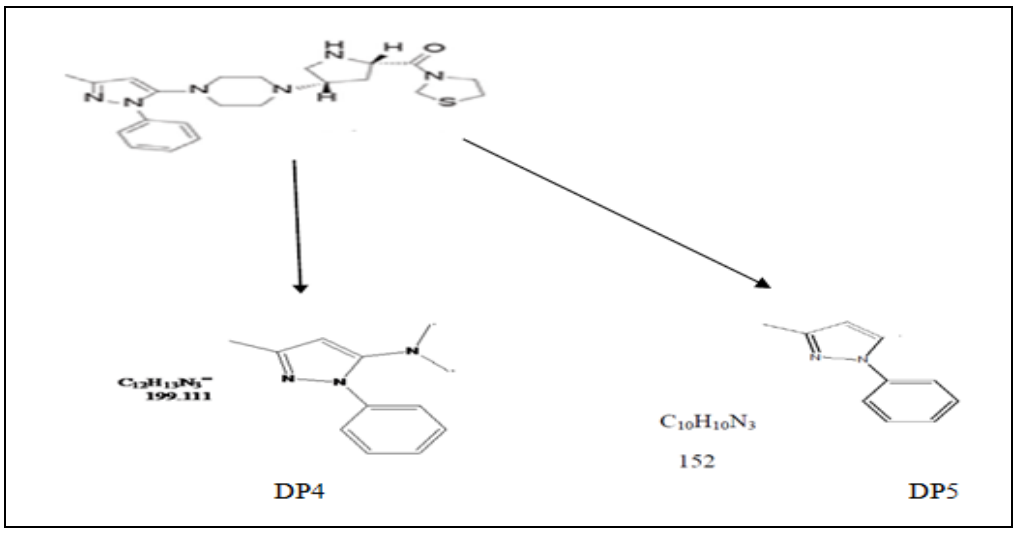

FIG. 14: DEGRADATION PATHWAY OF TENE IN ACID AS WELL AS ALKALINE STRESS CONDITION

CONCLUSION: The developed HPTLC methods for the simultaneous estimation of metformin hydrochloride and teneligliptin hydrobromide were found to be simple, accurate and precise and reproducible. The developed HPTLC method helps to separate drugs as well as all the degradation products of MET and TENE which proved to its stability-indicating nature. The use of the HPTLC method helps to separation and isolation of drug as well as degradation products and the LC-MS helps to identify the structure of each degradation product as a result we can understand the degradation pathway of the drug molecule. The stability-indicating the study of MET and TENE shows degradation in stress conditions. The drug degraded under acid, alkaline and oxidative, stress conditions, while it was found to be stable under Photolytic and thermal stress conditions. The developed HPTLC method was further used to study the degradation kinetics of MET and TENE in an alkaline medium as the degradation in the alkaline medium was found to be more as compared to the stress degradation condition. The % degradation of MET and TENE and the degradation rate constant of the drugs increase either temperature increases or strength of NaOH increases or both increase while the degradation half-life of the drug decrease either the strength of NaOH increases or temperature increases or both increase.

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REFERENCES:

1. ICH, (Q2R1), Harmonized Tripartite Guideline, Validation of analytical procedures: Text and Methodology, IFPMA. Geneva 2005; 1-13.
2. Patil MD, Bapna M, Shah P and Khoja SS: Development and validation of analytical method for simultaneous estimation of metformin hydrochloride and teneligliptin hydrobromide hydrate in the pharmaceutical dosage form. J Pharm Sci Bioscientific Res 2017; 7(2): 200-08.
3. Kumaraswamy G, Parashuram M and Pranay K: Development of a nove stability-indicating RP- HPLC method for simultaneous estimation of metformin hydrochloride and teneligliptin hydrobromide and in bulk and combined tablet dosage forms. Inn international J of Medical and Pharmaceutical Sciences 2017; 1; 2-7.
4. Patil D, Ahmad S, Shastry VM, Mujawar T and Thakare L: Analytical method development and validation for the simultaneous estimation of metformin and teneligliptin by RP-HPLC in bulk and tablet dosage forms. Journal of Pharmacy Research 2017; 11(6): 676-81.
5. Patil MD, Bapna M, Shah P and Khoja SS: Development and validation of analytical method for simultaneous estimation of metformin hydrochloride and teneligliptin hydrobromide hydrate in the pharmaceutical dosage form. J Pharm Sci Bioscientific Res 2017; 7(2): 200-08.
6. Sen AK, Hinsu DN, Sen DB, Zanwar AS and Maheshwari RA: Analytical method development and validation for simultaneous estimation of teneligliptin hydrobromide hydrate and metformin hydrochloride from its pharmaceutical dosage form by three different UV spectrophotometric methods. Journal of Applied Pharmacy 2016; 6(9): 157-65.
7. Kshirsagar SA, Mane SB, Yogesh S, Hanchate, Aniket S, Katte, Kaushik V and Kulkarni: UV spectrophotometric method development and validation for determination of

teneligliptin hydrobromide hydrate in api and in pharmaceutical dosage form. IJPRS 2018.
8. Yadav N and Goyal A: Method development and validation of teneligliptin in pharmaceutical dosage form by UV spectrophotometric methods. Inter J of Pharma Chemistry and Analysis 2017; 4(3): 54-8.
9. Yadav N and Goyal A: Method development and validation of teneligliptin in pharmaceutical dosage form by UV spectrophotometric methods. International Journal of Pharmaceutical Chemistry and Analysis 4(3): 54-58.
10. Kumari MV, Eswaramma P, Rao CN, Sravani VV, Thirupathamma B, Praveen K and Lokesh P: Analytical method development and validation of teneligliptin in pharmaceutical dosage form by RP-HPLC. European Journal of Biomedical AND Pharmaceutical sciences 2017; 4(6): 477-81.
11. Sunitha DPG, Karthikeyan R, Kumar BR and Muniyappan S: Quantitative estimation of teneligliptin by validated colorimetric and FTIR spectroscopic methods. World J of Pharmacy and Pharmaceutical Scien 2017; 6(8): 1680-85.
12. Shah DA, Agarwal K, Mehta AF and Patel VB: Stability indicating HPTLC method for the estimation of anti-diabetic drug teneligliptin. Current Pharmaceutical Analysis 2017; 3.
13. Shinde CV, Aher KB, Bhavar BG, Kakad JS and Chaudhari SR: Development and validation of UV spectrophotometric method and high-performance thin-layer chromatographic (HPTLC) method for estimation of teneligliptin hydrobromide in pharmaceutical preparation. Der Pharmacia Lettre 2016; 8(8): 291-01.
14. Shethi PD: High-performance thin-layer chromatography quantitative analysis of pharmaceutical formulations. New Delhi CBS Publishers 1996; 1-68.
15. Shrivastava M: High-performance thin-layer chromatography. New York Springer Heidelberg Dordr 2011; 27-29.
16. Burger K: Instrumental thin-layer chromatography/planner chromatography. UK Brighton Proceedings of the Interna Symposium 1989; 33-44.

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