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A STABILITY INDICATING UPLC METHOD FOR SIMULTANEOUS DETERMINATION OF TENELIGLIPTIN HYDROBROMIDE HYDRATE AND METFORMIN HYDROCHLORIDE IN BULK AND TABLET DOSAGE FORM

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Keywords:

Metformin Hydrochloride, Teneligliptin Hydrobromide, UPLC, Force Degradation Studies

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ABSTRACT: A stability indicating UPLC method was developed and validated for the determination of Teneligliptin Hydrobromide and Metformin Hydrochloride in bulk and tablet dosage form. The method was carried out using Endoversil column (2.1 × 50 mm, 1.8 µm) with mobile phase consisting of 0.1% v/v Ortho-phosphoric acid: Acetonitrile (80: 20) under isocratic mode with an injection volume 1 µL and at flow rate 0.3 ml/min and both the analytes were monitored at 221 nm. Stress conditions were performed by subjecting the individual analytes to the hydrolysis (acidic, basic), oxidation photolytic and thermal stress conditions. The retention times of metformin hydrochloride and teneligliptin hydrobromide were 0.443 and 0.694 min, respectively and showed a good linearity in the concentration range of 250 - 1250 µg/ml for metformin hydrochloride and 10 - 50 µg/ml for teneligliptin hydrobromide with a correlation coefficient of 0.9993 and 0.9990. The percentage recoveries of metformin and teneligliptin in the marketed dosage form found to be 99.86 and 99.96 respectively. The analytical performance of the proposed method was validated for specificity, linearity, accuracy, precision and Robustness, Detection and Quantitation limits. This method can be used for the routine estimation of these drugs in combined pharmaceutical dosage form.

INTRODUCTION: Teneligliptin hydrobromide hydrate (TEN) is chemically described as {(2S, 4S)- 4- [4- (3- methyl- 1phenyl- 1Hpyrazol- 5-yl) piperazin- 1- yl] pyrrolidin- 2- yl} (1, 3-thiazolidin- 3yl) methanone hemipentahydrobromide hydrate is a dipeptidyl peptidase inhibitor. It involves the inactivation of the incretin hormones (glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP).

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These incretin hormones are rapidly degraded by the enzyme DPP-4. Both incretin hormones are involved in the physiological regulation of glucose homeostasis. GLP-1 and GIP are secreted by the intestine at a low basal level throughout the day and concentrations are increased in response to a meal [5].

GLP-1 and GIP increase insulin biosynthesis and secretion from pancreatic beta cells in the presence of normal and elevated blood glucose levels. Teneligliptin binds to DPP-4 in a reversible manner and thus leads to an increase and a prolongation of active incretin levels. Teneligliptin glucose dependently increases insulin secretion and lowers glucagon secretion thus resulting in an overall improvement in the glucose homoeostasis.

Metformin hydrochloride (MET) is 1, 1-dimethylbiguanide hydrochloride, a biguanide antidiabetic. It is given orally in the treatment of type 2 diabetes mellitus and is the drug of choice in overweight patients [3]. They do not stimulate insulin release but require that some insulin be present in order to exert their antidiabetic effect. Possible mechanism of action includes the delay in the absorption of glucose from the GIT and increase in insulin sensitivity and glucose uptake in to cells and inhibition of hepatic gluconeogenesis. For effective control of blood sugar in diabetic patients more than one medication is required. TEN shows effective control of blood sugar when combined with MET. Chemical structures of both the drugs are shown in **Fig. 1**. Literature survey revealed that various analytical methods reported for the estimation of Metformin and Teneligliptin individually using UV Spectrophotometry, HPTLC and LC-MS/MS. Moreover, many methods were reported for the estimation of MET along with other drugs in combined formulation and TEN individually [2]. The development of simultaneous estimation of TEN and MET in combined dosage form has not yet been reported by any method.

Hence in the present work we aimed to develop a new, simple, fast, rapid, accurate, and precise, less time consuming UPLC method for the simultaneous determination of metformin and Teneligliptin in bulk and tablet dosage form. The proposed methods were validated according to ICH guidelines.

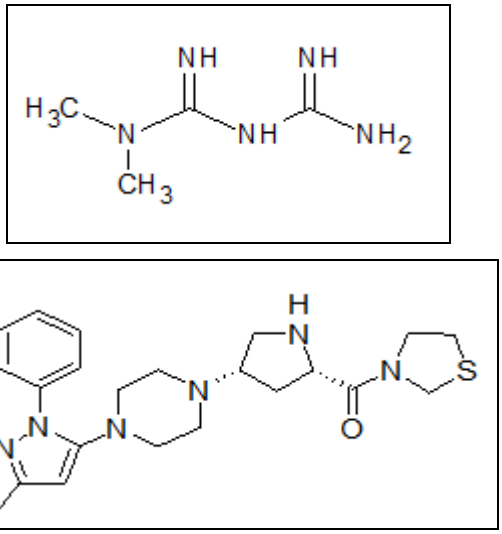

FIG. 1: CHEMICAL STRUCTURES OF MET (METFORMIN HYDROCHLORIDE) AND TEN (TENE-LIGLIPTIN HYDROBROMIDE HYDRATE)

Ultra Performance Liquid Chromatography Method: Experimental: Materials: TEN reference standard used throughout the experiment was received as gift sample from Dr. Reddy's Pharmaceuticals Ltd., and MET was obtained from Larus Laboratories, Telangana, India. The pharmaceutical formulation, Zeta met Plus® tablet (Glenmark Pharmaceuticals Ltd., Mumbai, India) containing 20 mg of TEN along with 500 mg of MET was purchased from commercial sources. Acetonitrile and Water (HPLC grade) were purchased from Merck Mumbai Ltd., India. All other chemicals and reagents employed were of analytical grade.

Instrumentation and Chromatographic Method: The analysis of the drugs was carried out on a UPLC system equipped with a reverse phase Endoversil (2.1 × 50 mm, 1.8 µm), Pump with 1 µl injection loop and a PDA detector running on Empower Software. The mobile phase consists of Acetonitrile: 0.1% OPA buffers (20:80 v/v) and the flow rate was maintained at 0.3 ml/min. The mobile phase was freshly prepared and passed through whatmann filter paper of pore size of 0.45 µm and it was degassed by sonicating for 5 min before it was used. The elution was monitored at wavelength of 221 nm with UV detector, and the injection volume was 1 µl.

Determination of Maximum Absorbance: The standard solutions of 10 µg/ml Teneligliptin hydrobromide hydrate and metformin hydro-chloride were scanned in the range of 200 - 400 nm against mobile phase as blank. From the UV spectrum wavelength of 221 nm was selected. At this wavelength metformin hydrochloride and teneligliptin standards show good absorbance.

Preparation of Standard Stock and Working Standard Solutions of Teneligliptin and Metformin: Preparation of Standard Stock Solution: Accurately weigh and transfer 125 mg of Metformin and 5 mg of Teneligliptin working standard into a 10 ml clean dry volumetric flask add about 7 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Preparation of Working Standard Solution: 0.6 ml from standard stock solution was pipette out and

taken into 10ml volumetric flask and made up with diluent (750 µg/ml of MET and 30 µg/ml of TEN).

Preparation of Sample Stock Solution: Ten tablets were weighed; average weight was calculated and crushed to obtain fine powder. Accurately weighed quantity of tablet powder equivalent to about 125 mg of Metformin and 5mg of Teneligliptin was transferred to a 10 ml clean dry volumetric flask, added 7ml of mobile phase and ultrasonicated for 20 min, volume was then made upto the mark with mobile phase. The solution was then mixed and filter through whatmann filter paper no. 42.

Preparation of Working Sample Solution: 0.6 ml from sample stock solution was pipette out and taken into 10 ml volumetric flask and made up with diluent (750 µg/ml of MET and 30 µg/ml of TEN).

Method Development: The UPLC method developed in this study was aimed at finding the chromatographic system capable of eluting and resolving teneligliptin hydrobromide hydrate, metformin hydrochloride and its degradation products with satisfying system suitability conditions.

To develop the conditions various parameters such as mobile phase, pH, Flow rate and solvent ratio were changed and suitable chromatographic condition has been developed for routine analysis of drug samples.

Initial trails were carried out by using Phenominex (2.1 × 50 mm, 1.8 µm) column taking water: Methanol in various proportions with flow rate of 0.3ml/min. The column was maintained with isocratic phase. The chromatograms obtained after injecting drug samples and maintained with run time of 9 min reported no peaks were observed. Further trails were carried out using different mobile phase of water: Acetonitrile in various proportions and reported the poor resolution in separation and peaks were observed broad with thick peak heads and high retention time. Then trails were carried out using different mobile phase of 0.1% OPA: Acetonitrile using same column and reported no proper resolution, proceeding with same mobile phase but column was changed Endoversil (2.1 × 100 mm × 3 µm) reported that peaks were eluted with tailing and no proper resolution. The best resolution was reported during a trail when mobile phase was taken as 0.1% OPA: Acetonitrile in the ratio of 80:20 with the flow rate of 0.3 ml/min, acidic pH of 3.2 in the solvent system and sharp peaks was depicted at retention time of 0.2 min and 0.4 min, peak was narrow, sharp and with high resolution compared to other peaks obtained in different trails. Thus, these chromatographic conditions were used for studying the different properties of drug such as degradedness and also used to validate various method parameters like linearity, precision, recovery, ruggedness, robustness, LOD and LOQ. Chromatographic condition was established such that it could be suitable for separation of drug and its degradation products separating impurities during elution from the chromatographic column.

The proposed method is simple, rapid and validated for its accuracy. No interfering peaks were found in the chromatograms indicating that the tablet excipients did not interfere in the analysis of drugs.

Method Validation: The Proposed method was validated according to ICH guidelines. The parameters assessed were linearity, precision, accuracy, Robustness, stability, LOD and LOQ.

Linearity: Linearity of the proposed method was evaluated according to the ICH guidelines by the analysis of working standard solution of Teneligliptin hydrobromide hydrate and Metformin hydrochloride at different concentrations ranging from 10 - 50 µg/ml and 250 - 1250 µg/ml. The linearity of an analytical procedure is the ability to obtain test results that are directly proportional to the concentration (amount) of an analyte in the sample within a given range. Linearity was evaluated by linear - regression analysis. Corresponding peak area values of different concentrations were determined and graph was plotted between concentration on x-axis and peak area values on y-axis.

Precision: The precision of the analytical method expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision is usually expressed in variance, standard deviation or coefficient of variance of a series of measurements [6].

Recovery: The accuracy of method was determined by recovery, by spiking of standard drug solution to pre analyzed sample at three different levels *i.e.,* at 50, 100, and 150%. The resultant solutions were then reanalyzed by the developed method. At each concentration, sample was injected thrice to check repeatability and from the data it was analyzed that the method was accurate [9].

Ruggedness: The degree of reproducibility obtained by analyzing the same sample under variety of normal test conditions such as different analyst, instrument, day, reagent columns *etc*. Comparison of reproducibility of test results to the precision of assay is the direct measure of ruggedness of the method [11].

Robustness: Robustness is the measure of analytical method to remain unaffected by small, deliberate variations in the method parameters. It provides its reliability during normal usage [9].

Limit of Detection: Limit of detection of the individual analytical method is the lowest concentration of analyte in the sample that the method can detect but not necessarily quantify under stated experimental conditions. LOD not only depend on procedure of analysis but also on type of instrument. LOD was calculated using the formulae, LOD = S/N where Average Baseline Noise obtained from Blank was named as (S), Signal Obtained from LOD solution (0.25% of target assay concentration) was named (N) [9].

Limit of Quantification: Limit of quantification of the individual analytical method is the lowest concentration of analyte in the sample, which can be quantitatively determined with suitable precision and accuracy under stated experimental conditions. The quantification limit is used particularly for the determination of impurities and degraded products. LOQ is calculated by the formulae, LOQ = S/N, where S was Average Baseline Noise obtained from Blank, N was Signal Obtained from LOD solution (0.75% of target assay concentration) [10].

Forced Degradation Studies: Stress degradation was carried out on the drug in order to check the stability of the drug by providing various stress conditions like light, heat, acid, base and oxidation compared against blank solution stored under normal conditions. The purpose of stability indicating assay method is to provide evidence that the analytical method is efficient in determination of drug substances in commercial drug product in the presence of its degradation products [12].

Preparation of Stock: Accurately weigh 10 tablets crush in mortar and pestle and transfer equivalent to 125 mg of Metformin and 5 mg Teneligliptin in sample into a 10 mL clean dry volumetric flask add about 7 mL of diluent and sonicate it up to 5 min to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.44 micron Injection filter (Stock solution) [14].

Acid Hydrolysis: Hydrolytic study under acidic and basic condition involves catalyzation of ionisable functional groups present in the molecule. HCl and NaOH are employed for generating acidic and basic stress samples, respectively. Pipette 0.6 ml of above solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 60 ºC for 48 hours and then neutralized with 0.1 N NaOH and make up to 10 ml with diluent. Filter the solution with 0.44 microns syringe filters and place in vials. The sample was injected after the system suitability solution has been injected and degradedness in the chromatogram was evaluated by comparing with initial values [16].

Base Hydrolysis: To study the base hydrolysis, Pipette 0.6 ml of above solution into a 10 ml volumetric and add 3 ml of 0.1N NaOH was added in 10 ml of volumetric flask. Then, the volumetric flask was kept at 60 ºC for 24 hours and then neutralized with 0.1N HCl and make up to 10 ml with diluent. Filter the solution with 0.44 microns syringe filters and place in vials. The drug solutions prepared earlier were injected into the UPLC column after system suitability solution has been injected and degradants in the chromatograms were evaluated and compared with initial values without base hydrolysis [16].

Photolytic Degradation: Photolytic degradation is carried out by exposing the drug substance (in solid as well as in the solution form) or drug product to a combination of visible and UV light. The most commonly accepted wavelength of light is in the

range of 300 - 800 nm to cause the photolytic degradation. To study the photolytic degradation of the drug sample, Teneligliptin hydrobromide hydrate and metformin hydrochloride was exposed to UV light and normal light. The drug was dissolved in diluent at zero hour and photolyzed sample was prepared by exposing the powdered drug form in Petri dish to lab light and UV light. After 48 hours, the powdered drug forms were added with diluents and solutions were injected into the chromatographic column. Chromatograms were evaluated for degradants and compared with initial values [15].

Thermal degradation in general, rate of a reaction increase with increase in temperature. Hence, the drugs are susceptible to degradation at higher temperature. Many Active pharmaceutical ingredients were sensitive to heat or tropical temperatures. Thermal degradation involves different reactions like pyrolysis, hydrolysis, decarboxylation, isomerization, rearrangement and polymerization. Thermal degradation study is carried out at 40 ºC to 80 ºC. The most widely accepted temperature is 70 ºC for thermal degradation. High temperature (>80 ºC) may not produce predictive degradation pathway. In order to study the thermal degradation, drug samples were kept in Petri dish and exposed to high temperature around 40 °C to 80 °C in hot air oven. After exposing the samples to high temperature for 48 hours, solutions were prepared and injected into the chromatographic column. After studying the chromatograms degradedness was evaluated and compared with initial values [15].

Oxidative Degradation: Many drug substances undergo autoxidation *i.e.*, oxidation under normal storage condition and involving ground state elemental oxygen. Therefore it is an important degradation pathway of many drugs. Autoxidation is a free radical reaction that requires free radical initiator to begin the chain reaction. Hydrogen peroxide, metal ions, or trace level of impurities in a drug substance act as initiators for autoxidation. Pipette 0.6 ml above stock solution into a 10 ml volumetric flask and 1ml of 12.5% w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials. The prepared drug samples were injected into the chromatographic column after system suitability solution has been injected; chromatograms were evaluated for degradants in the chromatograms and compared with initial values (without oxidation) [15].

RESULTS AND DISCUSSION

Assay:

TABLE 1: ASSAY

Drug	Label claim (mg)	Amount found	Drug content (%)
Metformin	500	499.85	99.97
Teneligliptin	20	20.128	100.64

Assay Results of Metformin Hydrochloride:

855999.7/854516.7 × 125/10 × 0.6/10 × 10/199.5 × 10/0.6 × 798/500 × 99.8/100 × 100 = 99.97%

Assay Results of Teneligliptin Hydrobromide:

115671/114706 × 5/10 × 0.6/10 × 10/199.5 × 10/0.6 × 798/20 × 99.8/100 × 100 = 100.64%

Linearity: The calibration curve showed **Fig. 1** and **2** good linearity in the range of 250 - 1250 µg/ml for metformin and 10 - 50 µg/ml for teneligliptin (API) with correlation coefficient (r^2) of 0.9993 for metformin and 0.9990 for teneligliptin. A typical calibration curve has the regression equation of y = 1202.7X – 59254 for metformin and y = 4409.5X – 17183 for teneligliptin Results are given in **Table 2**.

TABLE 2: CONCENTRATION *vs.* AREA

Drug	Metformin Hydrochloride		Teneligliptin Hydrobromide	
S. no.	Concentration (µg/ml)	Peak area	Concentration (µg/ml)	Peak area
1	250	244841	10	29672
2	500	525756	20	68336
3	750	856654	30	113345
4	1000	1150925	40	159680
5	1250	1435608	50	204473
Correlation coefficient (r^2)		0.9993		0.9990
Intercept		59254		17183
Slope		1202.7		4409.5

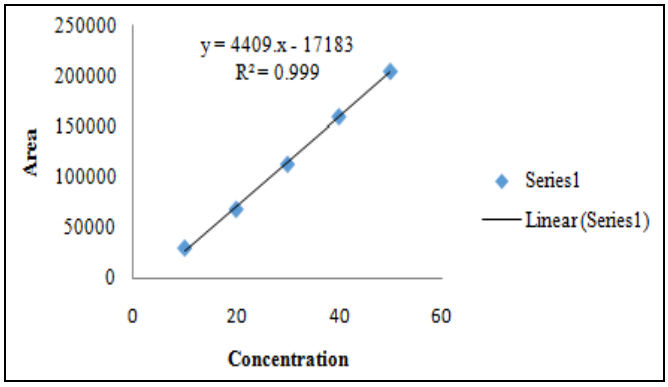

FIG. 2: LINEARITY RESULTS OF TENELIGLIPTIN HYDROBROMIDE HYDRATE

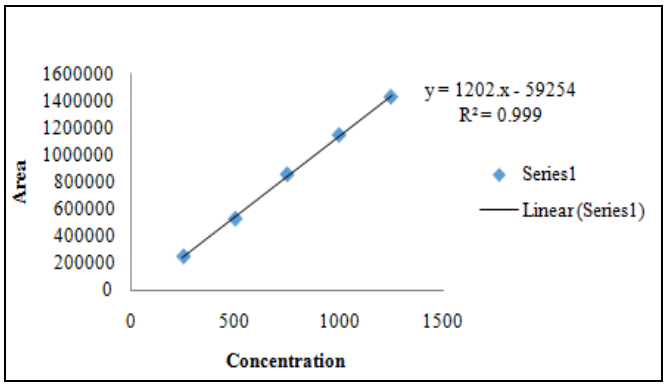

FIG. 3: LINEARITY RESULTS OF METFORMIN HYDROCHLORIDE

Precision: Intraday precision was carried out using test samples prepared and analyzed on the same day. The % RSD was found to be 0.4 for metformin and 0.8 for teneligliptin for intraday precision. The low % RSD values below 2 indicate that the method is precise. The results are given in **Table 3**.

TABLE 3: INTRADAY PRECISION OF METFORMIN AND TENELIGLIPTIN

	Metformin Hydrochloride			Teneligliptin Hydrobromide		
S. no.	Concentration (µg/ml)	Retention time (min)	Peak Area	Concentration (µg/ml)	Retention time (min)	Peak Area
1	750	0.422	852828	30	0.694	111368
2	750	0.423	852337	30	0.709	112717
3	750	0.424	858355	30	0.714	112655
4	750	0.425	852839	30	0.720	113939
5	750	0.429	858513	30	0.720	113013
6	750	0.430	857582	30	0.721	112282
	Average		855409.0	Average		112662.3
	SD		3024.5	SD		845.7
	% RSD		0.4	% RSD		0.8

Recovery: At each concentration, samples were injected thrice to check repeatability and from the RSD values it was analyzed that the method was accurate as % recovery values found to be in the range of 99.77% to 99.96% for metformin and 99.56% to 100.26% for teneligliptin.

TABLE 4: RECOVERY OF METFORMIN HYDROCHLORIDE

% Concentration (at specification Level)	Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	427928	62.5	62.47	99.96	99.86
100%	854989	125	124.82	99.86	
150%	1281399	187.5	187.07	99.77	

TABLE 5: RECOVERY FOR TENELIGLIPTIN HYDROBROMIDE HYDRATE

% Concentration (at specification Level)	Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	57620	2.5	2.51	100.26	99.96
100%	114986	5	5	100.04	
150%	171648	7.5	7.47	99.56	

Robustness: The deliberate change in the flow rate, Mobile phase composition, Temperature variation was made to evaluate the impact on the method.

Variation at flow rate (0.27 ml/min to 0.33 ml/min): The standard solution of Teneligliptin (30 ppm) and Metformin (750 ppm) was prepared and analyzed using various flow rates along with actual flow rate. On evaluation of the above results, it was concluded that the variation in flow rate did not affect the method significantly. Hence it indicated that the method was robust even by change in the flow rate ± 10% **Table 6** and **7**.

TABLE 6: SYSTEM SUITABILITY FOR METFORMIN

S. no.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.27	3221.82	1.10
2	0.3	2307.06	1.20
3	0.33	3315.17	1.10

TABLE 7: SYSTEM SUITABILITY FOR TENELIGLIPTIN

S. no.	Flow Rate (ml/min)	System Suitability Results		
		USP Plate Count	USP Tailing	USP Resolution
1	0.27	2311.07	1.15	3.12
2	0.3	3640.09	1.26	2.57
3	0.33	3757.93	1.11	2.98

*Results for actual flow (0.25 ml/min) have been considered from Assay standard.

Variation in Organic Composition of the Mobile Phase from 25% to 15%: The standard solution of teneligliptin (30 µg/ml) and metformin (750 µg/ml) was prepared and analyzed using the various mobile phase compositions along with the actual mobile phase composition in the method.

On evaluation of the above results, it was concluded that the variation in 10% organic composition in the mobile phase did not affected the method significantly. Hence it indicated that the method was robust even by change in the mobile phase ± 1 **Table 8** and **9**.

TABLE 8: SYSTEM SUITABILITY FOR METFORMIN

S. no.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3131.92	1.13
2	*Actual	2307.06	1.20
3	10% more	3309.54	1.31

TABLE 9: SYSTEM SUITABILITY FOR TENELIGLIPTIN

S. no.	Change in Organic Composition in the Mobile Phase	System Suitability Results		
		USP Plate Count	USP Tailing	USP Resolution
1	10% less	2975.18	1.09	4.35
2	*Actual	3640.09	1.26	2.57
3	10% more	6746.14	1.37	2.21

* Results for actual Mobile phase composition (80:20) Buffer pH 3: Acetonitrile has been considered from Accuracy standard.

Ruggedness / Intermediate Precision: The RSD values illustrates that method was suitable to analyze different drugs as values were in the order of repeatability depicting the precision of the method. In spite of changing the analyst the peak area values were reported in repeated manner thus showing the efficiency of the method and can be used to estimate various other drug samples using this method.

TABLE 10: TABLE SHOWING RESULTS OF METFORMIN AND TENELIGLIPTIN

	Metformin Hydrochloride			Teneligliptin Hydrobromide		
S. no.	Concentration (µg/ml)	Retention time (min)	Peak Area	Concentration (µg/ml)	Retention time (min)	Peak Area
1	750	0.443	859453	30	0.694	112535
2	750	0.445	857162	30	0.709	111224
3	750	0.424	859458	30	0.714	112915
4	750	0.425	858377	30	0.720	113391
5	750	0.429	858482	30	0.720	113108
6	750	0.430	859771	30	0.721	112959
	Average		858783.8	Average		112688.7
	SD		976.1	SD		769.7
	%RSD		0.1	%RSD		0.7

Limit of detection (LOD) and Limit of Quantification (LOQ): The LOD of metformin and teneligliptin was found to be 0.63 µg/ml and 0.44 µg/ml. The LOQ of metformin and teneligliptin was found to be 2.09 µg/ml and 1.43 µg/ml estimated by using the standard formulas.

The low values of LOD and LOQ illustrates that the developed method was sensitive, accurate and precise as it can detected and quantify with very low concentration.

Stability Indicating Studies: UPLC study of samples obtained on stress testing of metformin hydrochloride and teneligliptin hydrobromide hydrate under different conditions using mixture of 0.1% OPA: Acetonitrile in the ratio of 80:20 (v/v) as a mobile phase suggested following degradation behavior. All the samples were analyzed for purity peak of teneligliptin and metformin. In all the samples, Peak purity meets the acceptance limits (purity angle should be less than purity threshold).

TABLE 11: TABLE SHOWING FORCED DEGRADATION DATA FOR METFORMIN

S. no.	Degradation studies	Retention Peak	Area	Height	USP Plate Count	USP Tailing Factor	Purity Angle	Purity Threshold
1	Hydrolytic degradation under acidic condition	0.437	806691	84080	3295.45	1.13	0.152	0.527
2	Hydrolytic degradation under alkaline condition	0.433	809733	53368	3277.02	1.10	0.231	0.486
3	Thermal induced degradation	0.425	809294	44755	4310.98	1.19	0.273	0.370
4	Oxidative degradation	0.437	809509	72827	3221.23	1.12	0.182	0.328
5	Photolytic degradation	0.433	807294	53238	3627.21	1.32	0.314	0.476

TABLE 12: TABLE SHOWING FORCED DEGRADATION DATA FOR TENELIGLIPTIN

S. no.	Degradation studies	Retention Peak	Area	Height	USP Plate Count	USP Tailing Factor	Purity Angle	Purity Threshold
1	Hydrolytic degradation under acidic condition	0.760	107272	5112	3251.77	1.10	0.342	0.418
2	Hydrolytic degradation under alkaline condition	0.739	108687	2909	3616.16	1.18	0.352	0.536
3	Thermal induced degradation	0.727	109871	2336	4902.68	1.13	0.310	0.419
4	Oxidative degradation	0.760	108017	6626	3245.43	1.21	0.253	0.396
5	Photolytic degradation	0.739	106732	2323	3729.21	1.32	0.314	0.476

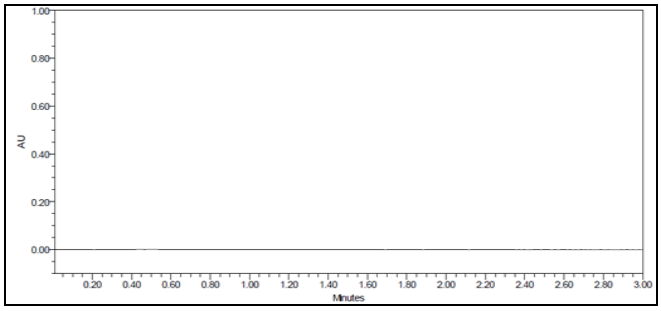

FIG. 4: CHROMATOGRAM OF BLANK SOLUTION

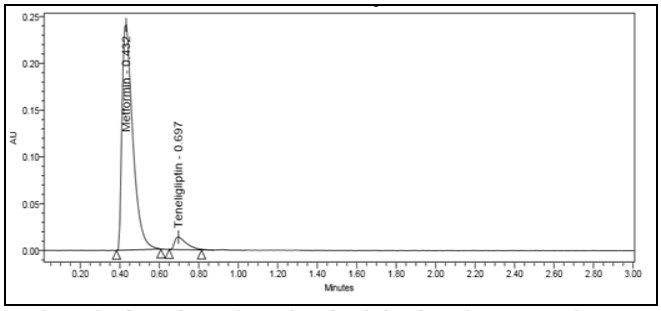

FIG. 5: STANDARD CHROMATOGRAM OF METFORMIN HYDROCHLORIDE AND TENELIGLIPTIN HYDROBROMIDE HYDRATE

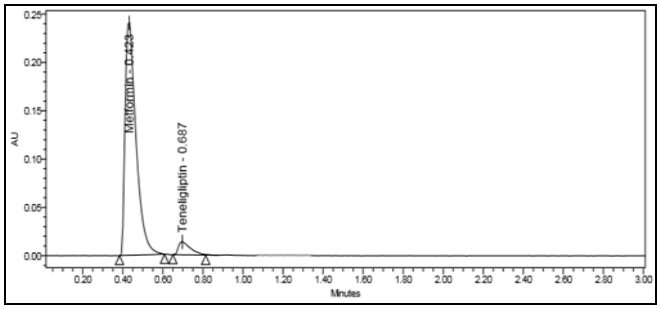

FIG. 6: SAMPLE CHROMATOGRAM OF METFORMIN HYDROCHLORIDE AND TENELIGLIPTIN HYDROBROMIDE HYDRATE

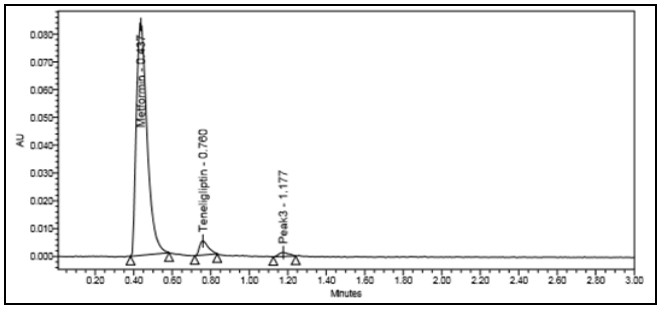

FIG. 7: ACID DEGRADATION CHROMATOGRAM OF METFORMIN AND TENELIGLIPTIN

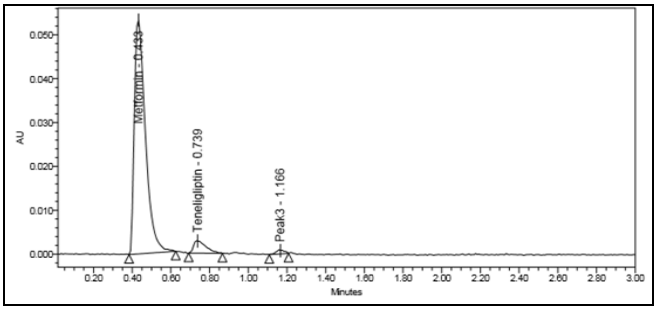

FIG. 8: BASE DEGRADATION CHROMATOGRAM OF METFORMIN AND TENELIGLIPTIN

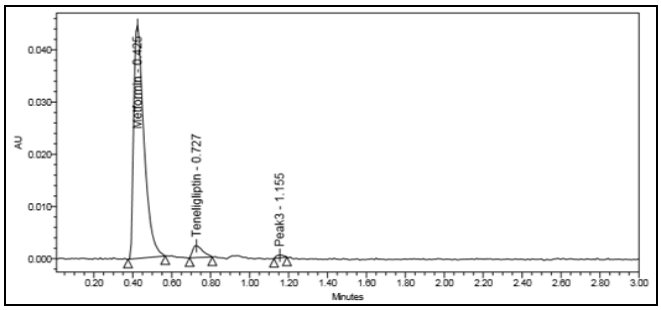

FIG. 9: THERMAL DEGRADATION CHROMATOGRAM OF METFORMIN AND TENELIGLIPTIN

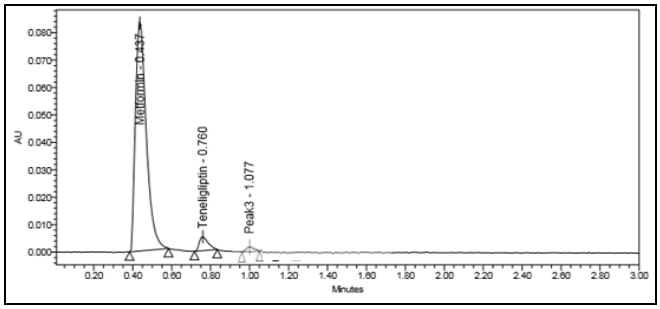

FIG. 10: OXIDATIVE DEGRADATION CHROMATOGRAM OF METFORMIN AND TENELIGLIPTIN

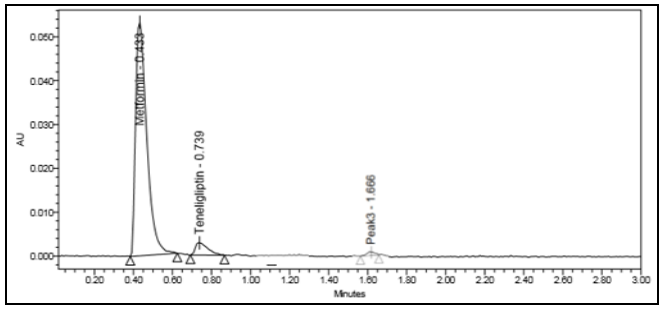

FIG. 11: PHOTO DEGRADATION CHROMATOGRAM OF METFORMIN AND TENELIGLIPTIN

CONCLUSION: A simple, precise, accurate, robust and cost-effective method was developed for the routine analysis. The method was successfully validated in terms of linearity, precision, accuracy as per ICH guidelines and it provides a linear response across a wide range of concentrations. Moreover, the method is fast with respect to analysis time compared to sophisticated chromatographic techniques. Hence it can be concluded that the proposed method was a good approach for obtaining reliable results and found to be suitable for the routine analysis, quality control and percentage degradation of pharmaceutical preparations containing these drugs either individually or in combination

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