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STABILITY-INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF TENELIGLIPTIN HYDROBROMIDE HYDRATE AND METFORMIN HYDROCHLORIDE IN A COMBINED TABLET DOSAGE FORM

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ABSTRACT
The proposed research palimpsest describes a simple, accurate, precise and reproducible RP-HPLC method for simultaneous estimation of Teneligliptinhydrobromide hydrate (TEN) and Metformin hydrochloride (MET) in a combined tablet dosage form. Separation was achieved on Shimadzu shimpack C_{18} column (250 mm × 4.6 mm, 5 µ) using 50mM potassium dihydrogen orthophosphate (KH_2PO_4) buffer pH 3: Methanol (40:60) as mobile phase, at flow rate of 0.8mL/min. The UV detection wavelength was 254 nm. The linearity was obeyed over a concentration range of 10µg/ml to 30µg/ml for Teneligliptin hydrobromide hydrate and 250µg/ml to 750µg/ml for Metformin hydrochloride with correlation coefficient below 0.999. The proposed method was validated for its accuracy, precision, robustness and system suitability parameters. The developed method was statistically evaluated and %RSD was found to be less than 2 throughout the validation. Forced degradation was performed by subjecting the drugs to various stress conditions like acid, alkali, oxidation, photolytic, humidity and thermal degradation. The method was successfully employed for the simultaneous estimation of Teneligliptinhydrobromide hydrate and Metformin hydrochloride in a combined tablet dosage form and validated as per ICH guidelines.

KEYWORDS: Teneligliptin hydrobromide hydrate, Metformin hydrochloride, RP-HPLC, Stability, Anti-diabetic.

INTRODUCTION
Teneligliptin hydrobromide hydrate, also known by a trade name of 'Tenelia' is a drug that treats type 2 diabetes mellitus. Teneligliptin hydrobromide hydrate {(2S, 4S)-4-[4-(3-methyl-1phenyl-1H-pyrazol-5-yl) piperazin-1-yl] pyrrolidin-2-yl} (1,3-thiazolidin-3-yl) methanonehemipentahydrobromide hydrate is a dipeptidyl peptidase (DPP-4) inhibitor.[1]

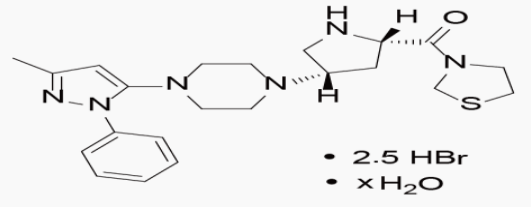

Fig. 1: Chemical structure of Teneligliptin hydrobromide hydrate (TEN).

The drug has an effective inhibition of DPP- 4 enzyme since Teneligliptin has a very distinctive J-shaped or anchor locked domain structurewhich provides potent and long duration of action.[2] Endocrine cells releases incretin hormones namely glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), and which leads to insulin secretion. Dipeptidyl peptidase-4 (DPP-4) rapidly inactivates incretine enzyme. Inhibition of DPP-4 activity leads to increase in the levels of active GIP and GLP-1. Thus, increased serum insulin levels and decreased serum glucagon levels in diabetic patients. Incretin-related agents such as DPP-4 inhibitors are therefore promising drugs in decreasing glucose fluctuations in patients with diabetes and have emerged as a new class of antidiabetic. Glycemic parameters are also handled with safety by Teneligliptin and also dose adjustments are just not required for renally impaired patients.[1] Metformin Hydrochloride (1,1-Dimethylbiguanide monohydrochloride) belongs to biguanide class of anti-diabetics.

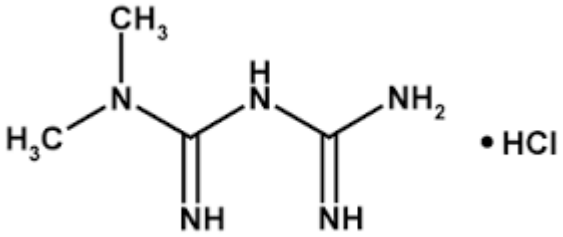

Fig. 2: Chemical structure of Metformin hydrochloride (MET).

Metformin do not cause insulin release but presence of some insulin is essential for their action. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization.[3] This agent helps reduce LDL cholesterol and triglyceride levels and prevents the cardiovascular complications of diabetes. Metformin is not metabolized and is excreted unchanged by the kidneys.

Literature review shows that, there are RP-HPLC methods developed for estimation of Teneligliptin hydrobromide hydrate and Metformin hydrochloride individually, for Metformin hydrochloride with other combination drugs and a very few RP-HPLC methods are present so far for simultaneous estimation of Teneligliptin hydrobromide hydrate and Metformin hydrochloride.[4,10] The literature survey also suggests that there is only one method so far developed for the degradation study of Teneligliptin hydrobromide hydrate and Metformin hydrochloride. Hence, there is a scope of betterment in developing a stability indicating RP-HPLC method for simultaneous determination of Teneligliptin hydrobromide hydrate and Metformin hydrochloride in a combined tablet dosage form.[11] So, an attempt was made to develop a simple, accurate and precise, stability-indicating RP-HPLC method for simultaneous determination of Teneligliptin hydrobromide hydrate and Metformin hydrochloride in a combined tablet dosage form.

MATERIAL AND METHODS

Materials

The active pharmaceutical ingredients of Teneligliptin hydrobromide hydrate and Metformin hydrochloride were obtained as a gift sample from Alkem Laboratories Ltd., Taloja, Raigad (TEN) and Aarti Drugs Ltd, Sarigam, Umbergaon (MET), respectively. Extended release tablet dosage form consisting of Teneligliptinhydrobromide hydrate (20mg) and Metformin hydrochloride (500mg) named Teneza-M 500, manufactured by Glenmark pharmaceuticals and marketed by Unichem Laboratories Ltd. was obtained. All other chemicals required for research was of analytical grade procured from Sigma Aldrich Chemicals Pvt Ltd and solvents for mobile phase were of HPLC grade and were procured from Thomas Baker Pvt Ltd.

Determination of maximum absorbance

The standard solutions of Teneligliptin hydrobromide hydrate were scanned in the range of 200-400 nm against diluent (80% methanol) as blank. The overlain spectra of Teneligliptinhydrobromide hydrate and Metformin hydrochloride against the diluent is shown in the **Fig. 3**.

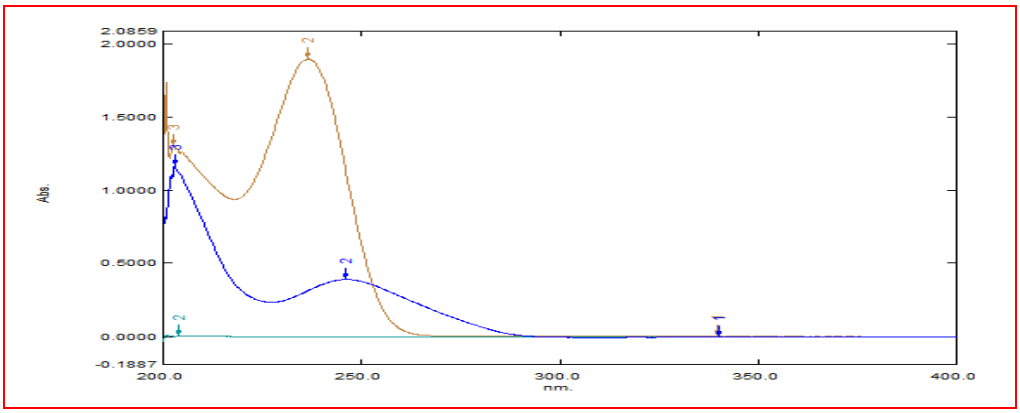

Fig. 3: Overlain spectra of Teneligliptin hydrobromide hydrate and Metformin hydrochloride.

Instrumentation and Chromatographic conditions

The research was carried out on Shimadzu Prominence I LC- 2030 HPLC using the software 'Lab solution'. The analysis of the drug was carried out on a Shimadzu shimpack C_{18} column (250 mm × 4.6 mm, 5 µ) column. The mobile phase consisted of 50mM potassium dihydrogen orthophosphate (KH_2PO_4) buffer pH 3: Methanol in a ratio of 40:60 v/v. The flow rate was maintained at 0.8ml/min. The UV detector's wavelength was monitored at 254 nm for the elution. The injection volume was 30µl and column oven temperature was maintained at 25°C.

Preparation of 50mM Potassium dihydrogen orthophosphate buffer (pH 3.0)

Accurately weighed 6.805g of Potassium dihydrogen orthophosphate, dissolved in 1 Litre of water and sonicated to dissolve. The pH was adjusted with o-phosphoric acid to 3.0 ± 0.05. The solution was filtered

using 0.45 µ membrane filter and degassed for 10 minutes.

Preparation of Diluent: 80% Methanol was used as a diluent.

Preparation of Mobile Phase
A mixture of 50mM Potassium dihydrogen orthophosphate buffer (pH 3.0) and Methanol in a ratio of 40:60 v/vwas used as mobile phase.

Preparation of standard stock solution of Teneligliptin hydrobromide hydrate
Stock A(TEN 1000 µg/ml)
About 25mg of TEN was accurately weighed and transferred into 25 ml volumetric flask respectively. The solution was then sonicated for 10 minutesto dissolve and diluted up to the volume with the diluent and filtered through 0.45µ membrane filter.

Stock B(TEN 200 µg/ml)
Pipette out 10 ml of Stock solution A and transferred into 50 ml volumetric flask respectively. The solution was sonicated for 10 minutes and diluted upto the volume with diluents. The stock solution was stored at 2-8 ºC.

Preparation of standard stock solution (MET 1000 µg/ml)
About 100 mg of MET was accurately weighed and transferred into 100 ml volumetric flask respectively. The solution was then sonicated for 10 minutes to dissolve and diluted up to the volume with the diluent and filtered through 0.45µ membrane filter. The stock solution was stored at 2-8 ºC.

Preparation of standard solution (TEN 20µg/ml and MET500µg/ml)
About 1ml of standard stock solution B of TEN and 5 ml of standard stock solution of MET was transferred into 10 ml volumetric flask and diluted up to the volume with the diluent.

Preparation of sample solution (TEN 20µg/ml and MET500 µg/ml)
Twenty tablets, each containing 20 mg of Teneligliptin and 500 mg of Metformin were weighed and finely powdered using mortar. A quantity of powder equivalent to 20 mg of Teneligliptin and 500 mg of Metformin was weighed and transferred to a 100ml volumetric flask. About 70 ml of diluent was added into the flask and sonicated for 20 minutes and diluted upto the volume with the diluent. The solution was filtered through 0.45µ membrane filter.1 ml of the filtered solution was added into a 10 ml volumetric flask and diluted upto the volume with the diluent to get a final concentration of 20 µg/mL of Teneligliptin and 500 µg/mL of Metformin.

Method validation[12,13]
Specificity
One of the considerable features of HPLC is its propensity to produce signals free from interference. Specificity refers to the power of the analytical method to differentiate and quantify the analyte in complex mixtures. An investigation of specificity is conducted during the validation of method and determination of impurities by injecting blank and placebo solution, to ensure the absence of interference from blank and placebo which are likely to be present in drug product.

Linearity
The linearity of a method is measured to see how well a calibration plot of response vs. concentration approximates a straight line. Suitable aliquots of the standard stock solutions of TEN (200 µg/ml) and MET (1000 µg/ml) were transferred into a series of 10 mL volumetric flasks to get concentration levels of 50, 80, 100, 120 and 150% (5 points) of standard concentration. Final volume was made up with the diluent. Each mixed standard solution was injected and chromatograms were recorded. The linearity of analytical curves was plotted for peak area of each drug against concentrations of drug.

LOD and LOQ
Limit of detection and limit of quantitation for both the drugs were estimated using the linearity data.

Precision
System precision was performed by injecting six replicates of a standard drug solution. Method precision was performed by injecting six different sample solutions prepared from single batch of tablet.

Accuracy
To check the accuracy of the proposed method, recovery studies were carried out at 80, 100 and 120% of the test concentration according to ICH guidelines. The recovery study was performed three times at each level.

Robustness
Robustness of the method was studied by deliberately changing the column oven temp (± 5^0C), mobile phase flow rate (±0.1 ml/min) and organic phase composition (±10%). The effect was studied in terms of various system suitability parameters like retention time, resolution, theoretical plates and tailing factor for both the drugs.

System suitability
The System Suitability was calculated from different parameters likeretention time, theoretical plates, resolution, tailing factor, limit of detection (LOD) and Limit of quantitation (LOQ).

Forced Degradation Study
Stability study was performed by treating the drug sample with different degradation conditions. Specificity

of the method was determined by checking interference of any of the possible degradation products produced during the forced degradation study. The forced degradation was carried out with 1 M HCl, 1 M NaOH, 5% v/v hydrogen peroxide, thermal (100°C) andphotolysis (365 nm) for finding out the stability nature of the drugs. After the fixed time period the treated drug sample were processed as perthe proposed sample preparation method. The specific stress conditions are described as follows.

Acidic degradation

Acidic degradation was carried out by adding 5 mL of 1 N HCl to the sample and after 1hr of heating at 80°C neutralizing the mixture by adding 1 N NaOH.

Alkali degradation

Alkali degradation was carried out by adding 1.0 mL of 1 N NaOH to the sample and after 1hr of heating at 80°C neutralizing the mixture by adding 1 N HCl.

Oxidative degradation

Oxidative degradation was performed by exposing the drug to 5 mL of 5% (v/v) H_2O_2 for 1 hr.

Thermal degradation

Thermal degradation was performed by heating the drug at 90ºC in oven for 1 hr.

Photolytic degradation

Photolytic degradation was carried out by exposing the drug to UV light (365 nm) inside UV chamber for 5 hr.

RESULT AND DISCUSSION

Method development and optimization

Method development process was carried out by examining different conditions like flow rate (0.8 mL/min, 1.0 mL/min and 1.2 mL/min), mobile phase compositions like acetonitrile: water, acetonitrile: buffer, methanol: water, methanol: buffer with different ratios were used. Both the drugs Teneligliptin hydrobromide hydrate and Metformin hydrochloride were found showing a significant UV absorbance at 254 nm, so this wavelength was chosen for UV detection. By use of a C18 column it was found the mobile phase consisting of 50mM potassium dihydrogen orthophosphate (KH_2PO_4) buffer pH 3: Methanol (40:60) provided well defined peak shape with good resolution. Metformin hydrochloride was eluted at about 3.1 min where as Teneligliptinhydrobromide hydrate eluted at 5.6min. The representative chromatograms of pure drug and combined drug product are shown in **Fig. 4-7** respectively.

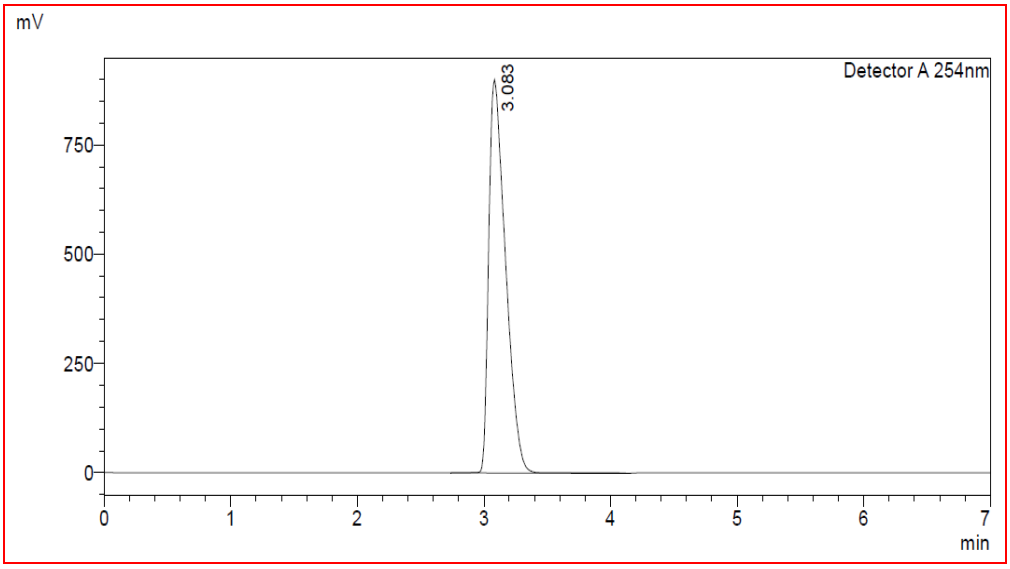

Fig. 4: Chromatogram for MET.

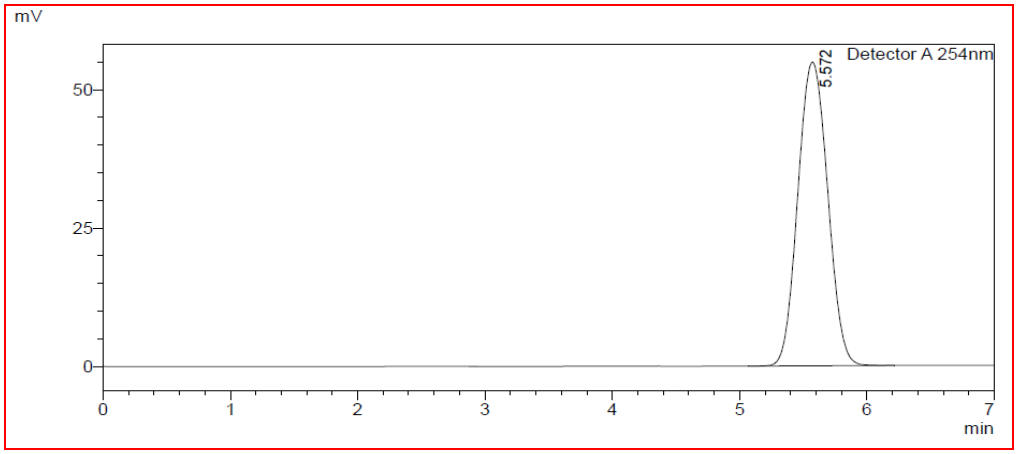

Fig. 5: Chromatogram for TEN.

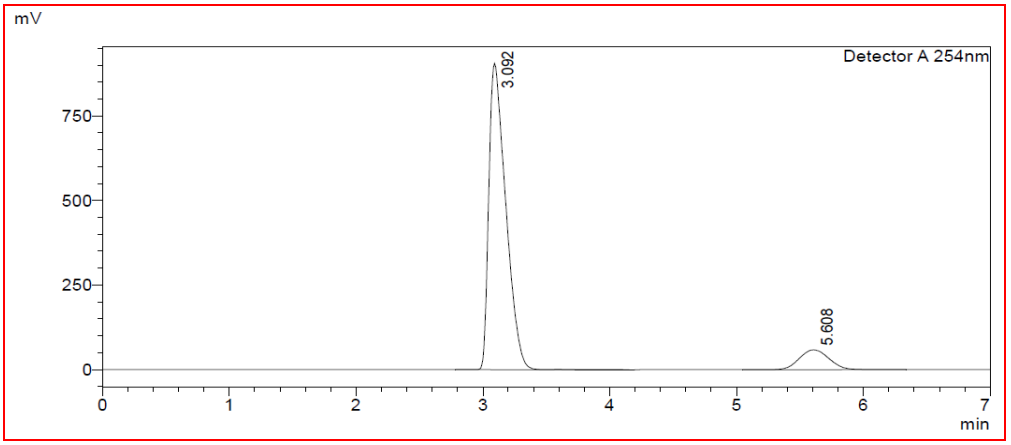

Fig. 6: Chromatogram for standard solution.

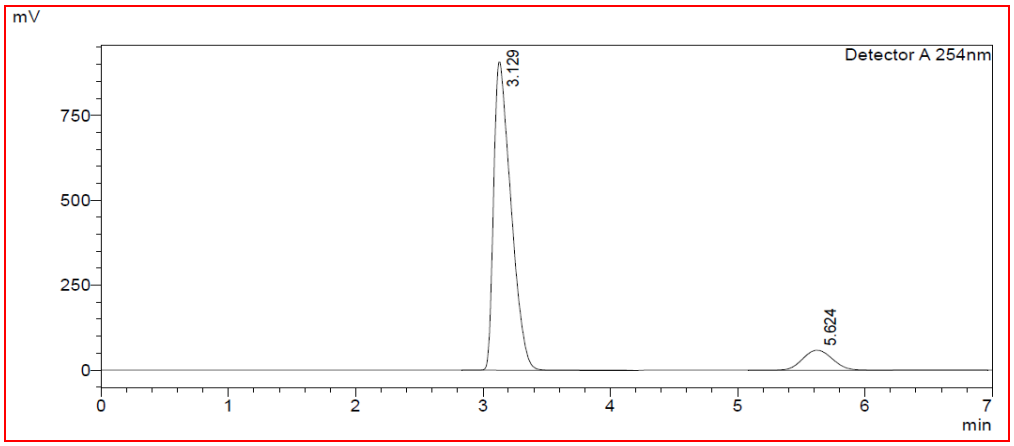

Fig. 7: Chromatogram for sample solution.

Method validation

Specificity

No interference was found in blank solution at the retention time ofTeneligliptin hydrobromide hydrate and Metformin hydrochloride as shown in **Fig. 8**. Thus the method was found to be specific.

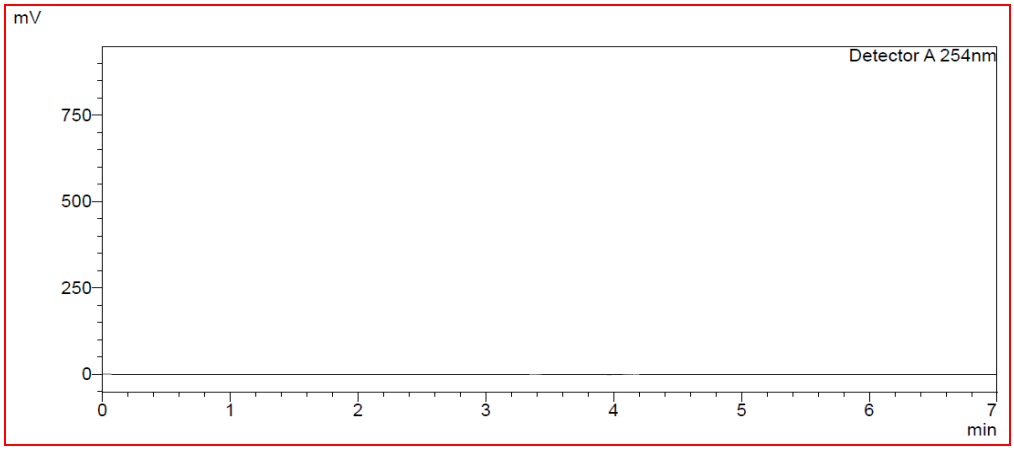

Fig. 8: Chromatogram for blank solution.

Linearity

Teneligliptin hydrobromide hydrate and Metformin hydrochloride have linearity over concentrations ranging from 0.5 to 150.0µg/ml. The slope (a) and intercept (b) were found to be 46025 and 20045.641 for Teneligliptin hydrobromidehydrate and 13973 and 1954477.514 for Metformin hydrochloride. Correlation coefficient was found to be 0.998 and 0.997 for both the drugs respectively. These results suggest a good linear relationship between peak area and analytes in the range studied. The linearity plots are as shown in **Fig. 9** and **Fig. 10**.

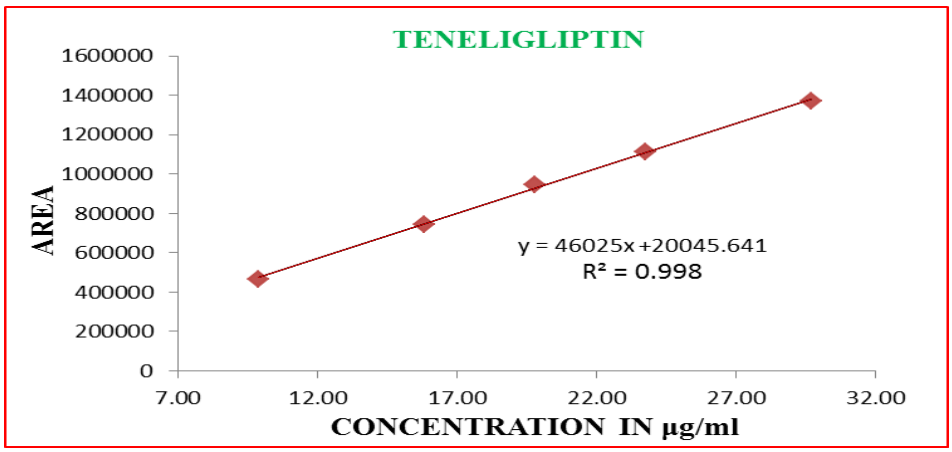

Fig. 9: Linearity plot for TEN.

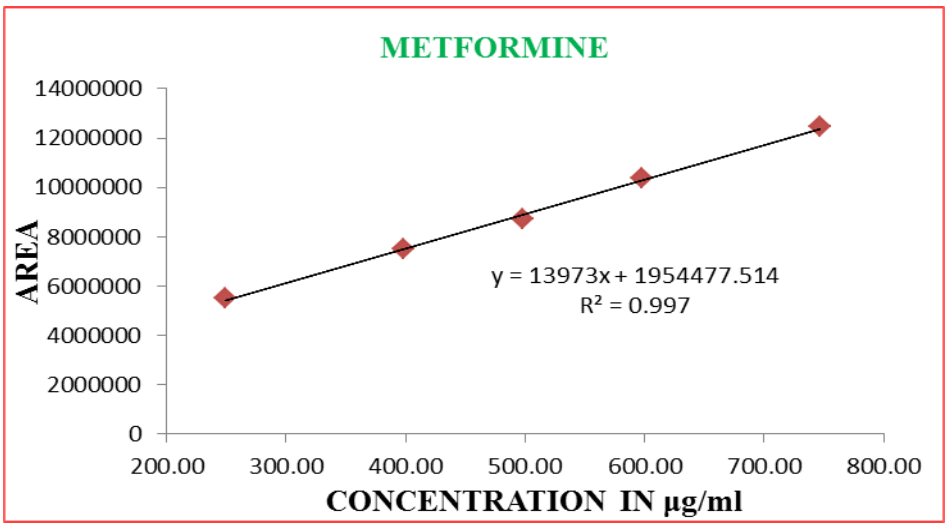

Fig. 10: Linearity plot for MET.

Precision

The RSD for method precision, systemprecision and intermediate precision were found to be less than 2% showing high degree of preciseness as shown in **Table 1**.

Table 1: Precision of method.

Sr.No.	Precision	TEN (%RSD)	MET(%RSD)
1	System Precision	0.926	1.269
2	Method Precision	0.402	1.197
3	Intermediate Precision	0.442	0.936

Accuracy

Initially a standard solution was analyzed and then accuracy was determined at three concentrations 80%, 100% and 120%. The amount found was compared with the amount added for preparing these concentrations and the recovery was found as shown in **Table 2**.

Table 2: Accuracy of method.

Sr.No.	Level	% Recovery	
		TEN	MET
1	80%	100.0	99.0
2	100%	100.0	100.3
3	120%	101.8	101.2

Robustness

The method was found to be robust under deliberate changes made in mobile phase flow rate and composition of organic phase. The results for robustness parameters are presented in **Table 3**.

Table 3: Robustness of method.

Sr.No.	Parameter		TEN		MET	
			Retention time (min)	Area	Retention time (min)	Area
1	Flow rate (ml/min)	0.7	5.697	995134	3.180	8559743
		0.9	5.569	982519	3.058	8490016
2	Temperature (ºC)	20	5.602	972001	3.157	8537491
		30	5.611	974561	3.133	8622109
3	Methanol (ml)	59.9	5.699	992226	3.164	8542012
		60.1	5.682	989736	3.171	8479023

System suitability

A critical evaluation of the method was performed. A higher resolution value indicates better separation of both the drugs. Also the LOD and LOQ values show superior sensitivity of the method. The system suitability parameters are shown in **Table 4**.

Table 4: System suitability of method.

Sr.No.	Parameters	TEN	MET
1	Retention time (min)	5.6	3.1
2	Tailing Factor	1.7	1.0
3	Theoretical plate	5434	3571
4	Resolution	7.1	---
5	LOD (µg/ml)	22.633	578.572
6	LOQ (µg/ml)	75.443	1928.573

Forced degradation studies

No interference because of excipients or degradation products produced after subjecting Teneligliptin hydrobromide hydrate and Metformin hydrochloride to forced degradation. No extra peaks were obtained either from the excipients used in the drug product or from the stress conditions applied on the drugs and drug product. Therefore the method was found specific and stability-indicating. Teneligliptin hydrobromide hydrate undergoes significant degradation in alkaline(36.1%) and oxidation(22.6%) condition whereas moderate degradation in acidic(9.99%) condition. Metformin hydrochloride undergoes significant degradation in alkaline(26.9%) condition whereas moderate degradation in acidic (4.5%) and oxidation(5.0%) condition. Both the drugswere found to be stable in thermal and UV stress condition. Degradation study results are shown in the **Fig. 11-15** and **Table 5**.

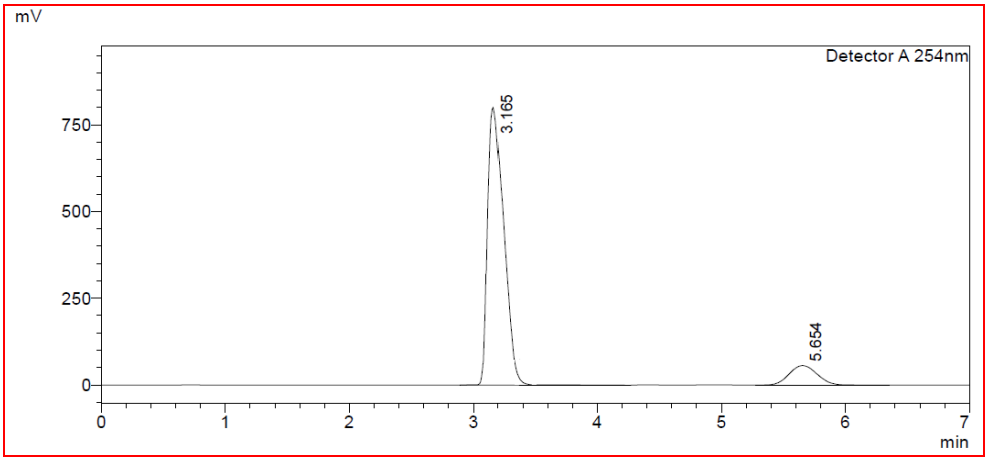

Fig. 11: Acid degradation.

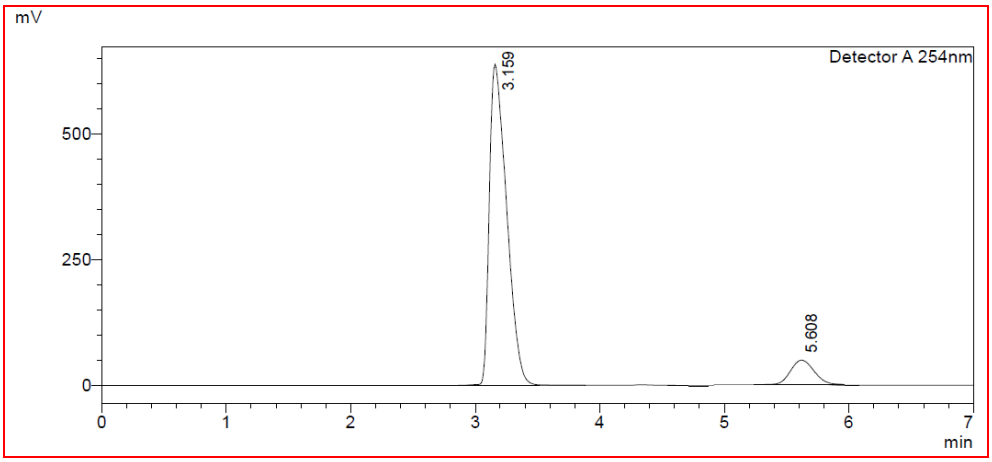

Fig. 12: Alkali degradation.

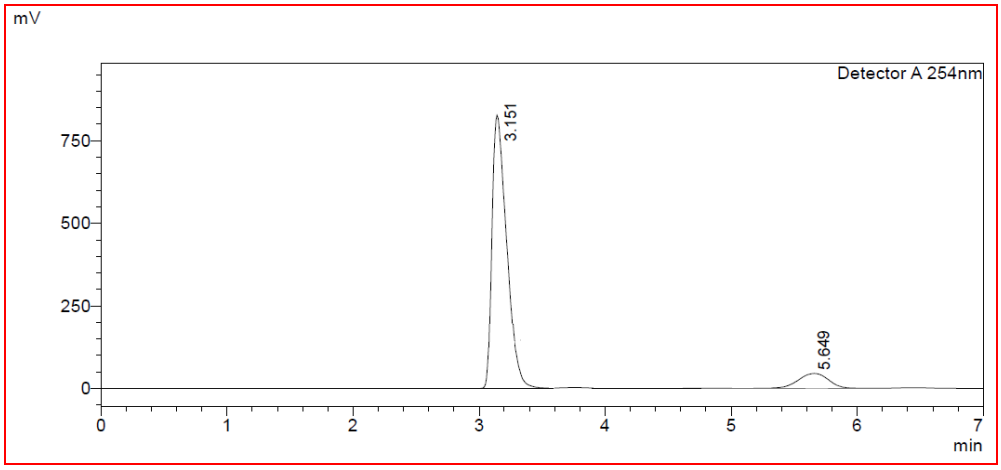

Fig. 13: Oxidative degradation.

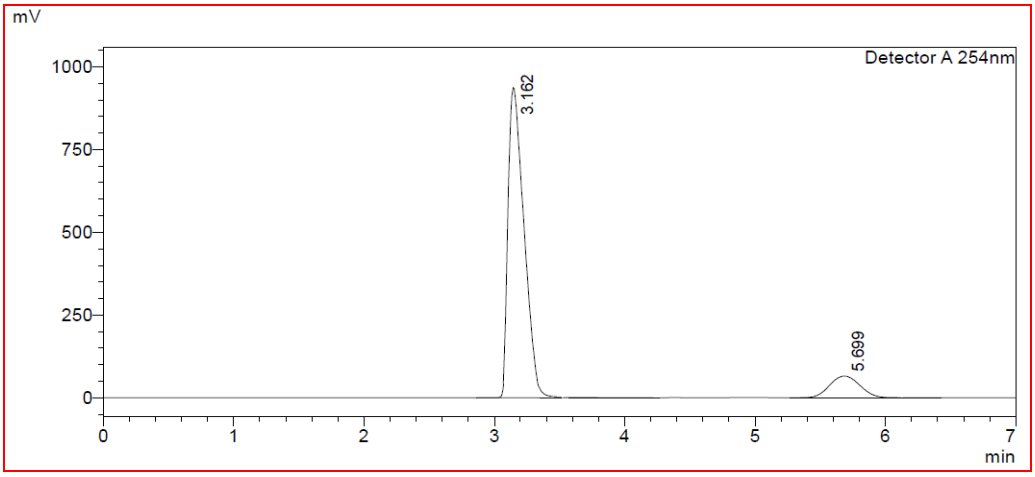

Fig. 14: Thermal degradation.

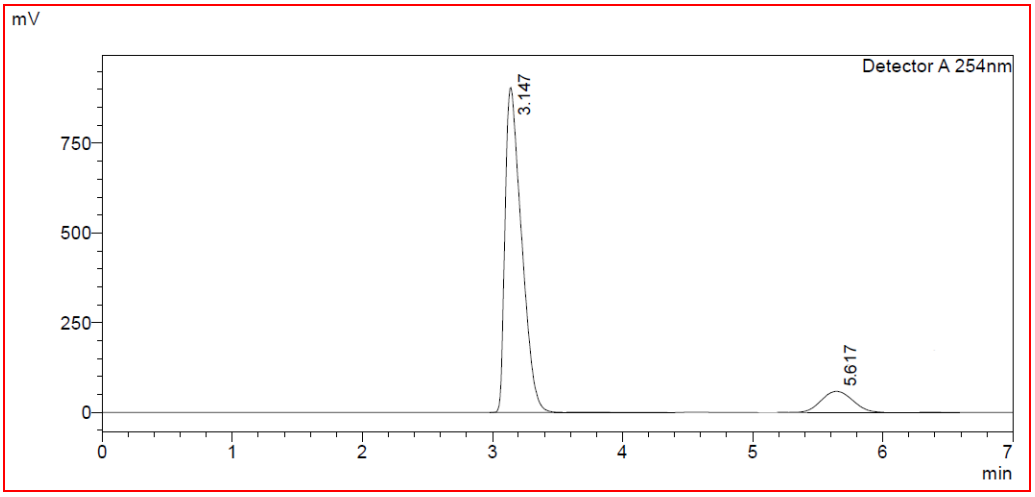

Fig. 15: Photolytic degradation.

Table 5: Forced degradation study results.

Condition	TEN		MET	
	%Assay	%Degradation w.r.t untreated sample	%Assay	%Degradation w.r.t untreated sample
Untreated Sample	100.2	---	99.5	---
Acid degradation	90.21	9.99	95.0	4.5
Base degradation	64.1	36.1	72.6	26.9
Peroxide degradation	77.6	22.6	94.5	5.0
Thermal degradation	98.4	1.8	100.2	-0.7
UV degradation	100.1	0.1	99.9	-0.4

CONCLUSION

This simple and accurate RP-HPLC method has been developed and validated for determining Teneligliptin hydrobromide hydrate and Metformin hydrochloride in a combined tablet dosage form. Lack of much study on degradation of both the drugs in a combined tablet dosage form is the driving cause for development of this method. The sample preparation technique is also very simple thus making it suitable for routine laboratory testing. The mobile phase is simple to prepare. Major advantage of this method is the analytes do not interfere with each other's elution with a good difference in retention times and goof peak shapes. Forced degradation ensures that the method is specific and able to describe the stability nature of both the drugs in a combined drug formulation. This degradation study can be incorporated for extensive study of degradation pathway of both the drugs at industrial level. Results of precision study demonstrate the superior preciseness of the method as the RSD values were well within the limits. The recovery value near 100% shows higher level accuracy of the method. So it can be concluded that the developed RP-HPLC method is novel, simple, accurate, precise, sensitive, and stability-indicating and can be employed successfully for the simultaneous determination of Teneligliptin hydrobromide hydrate and

Metformin hydrochloride in combined tablet dosage form.

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