RESEARCH ARTICLE

Development of Validated Stability-indicating Chromatographic Method for the Determination of Metformin and Teneligliptin and its Related Impurities in Pharmaceutical Tablets

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ABSTRACT

A simple, economical, precise, and selective reverse gradient phase high-performance liquid chromatography (RP-HPLC) method has been validated and developed to estimate related impurities of metformin and teneligliptin in a combined tablet dosage form. A RP-HPLC analysis was performed on Hypersil BDS C_{18} column, and its size was 250 mm X 4.6 mm, 5 µm with using mobile phase Acetonitrile and 0.05M potassium dihydrogen Phosphate with buffer pH-4.0 in the ratio of (20:80) at 225 nm detection wavelength with the flow rate of 1.0 mL/min. The analytical method was validated according to International Council for Harmonisation (ICH) guidelines. The linearity was observed in the Limit of Quantitation (LoQ)-37.5µg/ml range for Metformin and its related impurity A. Similarly, the LoQ-1.5 µg/mL range was observed linearity for Teneligliptin and its related impurity B. The correlation coefficient was more than 0.990 for both metformin and its related impurity A and teneligliptin and its related impurity B. The %recovery value was found to be a minimum of 96.181% and a maximum of 102.816% for metformin Impurity A. Similarly, the %recovery value was found to be a minimum of 96.999% and a maximum of 103.824% for teneligliptin impurity B. The relative standard deviation value for repeatability, interday precision, and intraday precision was less than 5%. The Limit of Detection (LoD) value was found to be 0.940 µg/mL for Metformin and 0.206 µg/mL for its related impurity A. The LoD value was found 0.038 µg/mL for Teneligliptin and 0.009 µg/mL for its related impurity B. The LoQ value was found at 2.849 µg/mL for Metformin and 0.623 µg/mL for its related impurity A. The LoQ value was found 0.116 µg/mL for Teneligliptin and 0.026 µg/mL for its related impurity B. The proposed method was found to be specific, linear, sensitive, precise, accurate, and robust in nature.

Keywords: ICH guidelines, Impurities, Metformin, Stability-indicating RP-HPLC, Teneligliptin, Validation.

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INTRODUCTION

Globally, about 463 million adults are living with diabetes. By 2045 this will rise to 700 million. The proportion of people with type 2 diabetes (T2D) is increasing in most countries. Diabetes caused 4.2 million deaths.[1] Diabetes mellitus type 1 is a disease caused by the lack of insulin secretion, and type 2 diabetes mellitus (T2DM) is a disease caused by insulin resistance by cells. Anti-diabetic drugs are used to treat diabetes mellitus by reducing the glucose level in the blood,[2] and the T2DM, which is characterized by polyphagia, polyuria, and polydipsia and needs a lifetime treatment with ant diabetic drugs.[3] The treatment goals involve reducing glycemic control and diabetes-associated cardiovascular risk. Hyperglycemia is associated with diminished life expectancy and quality due to microvascular and microvascular complications.[4] Patients suffering from the onset of diabetes are treated with insulin sensitizer and Metformin. The hypoglycemia risk is insignificant with Metformin treatment, drug interactions are less making, and it is a highly acceptable and safe first-line drug for the treatment of early-stage T2DM.[5] The etiology of T2DM is multiplex which involves several organs, and its treatments with different mechanisms of action by using a combination of drugs that effectively control the plasma glucose levels.[6]

Metformin hydrochloride (MET) is chemically N, N-dimethyl imidodicarbonimidic diamide hydrochloride (1, 1dimethylbiguanide hydrochloride). Metformin is an effective biguanide ant diabetic agent that has been used to control blood glucose levels of T2D patients for decades and has been considered the first line treatment according to international guidelines. Mitochondrial inhibition and activation of AMPK

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are key molecular effects of Metformin to inhibit hepatic gluconeogenesis. Metformin, on the other hand can, directly and indirectly, improve skeletal muscle sensitivity towards insulin.[7,8] It is official in Indian Pharmacopoeia,[9] British Pharmacopoeia,[10] European Pharmacopoeia,[11] and United States Pharmacopeia.[12]

Teneligliptin, chemically, [(2S, 4S)-4-[4-(5-methyl-2phenylpyrazol-3-yl) piperazin-1-yl] pyrrolidin-2-yl]-(1, 3thiazolidin-3-yl) methanone belongs to the class of anti-diabetic drugs known as dipeptidyl peptidase-4 inhibitors and used for the treatment of T2DM.[13,14]

Impurity profiling is the common name of a group of analytical activities, the aim of which is the detection, identification/structure elucidation, and quantitative determination of identified and unidentified (organic and inorganic impurities, residual solvents) impurities in bulk drugs and pharmaceutical formulations.[15,16] Since this is the best way to characterize the quality, safety, efficacy, and stability of bulk drugs and pharmaceutical formulations, this is the core activity in modern drug analysis. Impurity profiling is very crucial and critical during the synthesis of drug substances and manufacture of dosage forms, as it can provide crucial data regarding the quality, safety, efficacy, toxicity of drugs, various LoDs and LoQs, structures of several organic and inorganic impurities, usually associated with bulk drugs and finished products.

For the quality control aspects, it is essential to develop analytical methods for such combination products along with their impurities.[17] Various sophisticated analytical techniques are described in the literature to analyze Metformin and Teneligliptin either individually or in combination with other drugs by UV spectrometry method,[18,19] HPLC,[20-27] stability-indicating HPLC method.[28-30] However, the RP-HPLC stability-indicating chromatographic method for determining Metformin and Teneligliptinin with its related impurities in the dosage form is unavailable. So Precise, accurate, and sensitive method for stability-indicating chromatographic method for the determination of metformin and teneligliptin with its related impurities was planned and validated as per Q2 (R1) guideline.

EXPERIMENTAL

Materials

The Reference standard of metformin and its related impurity A, as well as teneligliptin and its related impurity B, were obtained as a gift sample from Cadila Pharmaceuticals Limited, Dholka, Ahmedabad. Methanol, Water, HPLC grade of Acetonitrile, and analytical reagent grade of Ortho phosphoric acid from Merck company Mumbai, were used for the study. And the commercially available tablet formulation of Metformin HCl and Teneligliptin, Tenalifine M (Healing Pharma India ltd.) with label claim Metformin HCl 500 mg and Teneligliptin 20 mg was procured from the market for use (Figure 1).

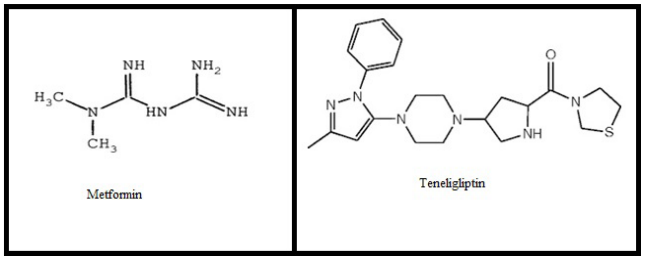

Figure 1: Chemical structure of metformin and teneligliptin

Instrumentation

Shimadzu LC-20 AT HPLC chromatographic system, Shimadzu digital weighing balance (ATX 224), lab Scientific Pvt. Ltd pH meter, Frontline Ultrasonic Cleaner ultra-Sonicator, India hot air oven, and Thermolab Mumbai, were used for the method development. A 0.45µ Millipore filter was used for filtration.

Chromatographic Conditions

The separation of Metformin HCl and Teneligliptin was achieved by using BDS Hypersil C_{18} column (250 mm X 4.6 mm, 5 µm), Acetonitrile: Buffer (pH 4.0): (20:80%, v/v) with the 1 mL/min flow rate, with an injection volume of 20.0 µL, at λ max of 225 nm, and runtime 15 minutes.

Preparation of Mobile Phase

The Potassium Dihydrogen Phosphate was Prepared with 0.05M concentration by dissolving accurately weigh of 6.8g of Potassium Dihydrogen Phosphate in 1000 ml HPLC grade water in a 1L volumetric flask and pH was adjusted to pH 4.0 with o-Phosphoric acid (OPA). The prepared buffer pH was checked by using a pH meter by ultra sonicating. For 5 minutes, the solution was degassed, and the obtained solution was filtered through a 0.45µ Millipore filter. And the mobile phase is prepared with the ratio of buffer (pH 4.0): Acetonitrile (80:20 v/v).

Standard Solutions Preparation

Preparation of Standard Stock Solution of Metformin (25 ppm):

Prepared the standard stock solution of Metformin by accurately weighing 25 mg of Metformin bulk drug into a 100 mL volumetric flask and dissolved in methanol, and it make up to the mark to get concentration of 250 µg/mL of Metformin Standard Stock Solution. For the preparation of 25µg/ml (25 ppm) of Metformin Working Standard Solution. The Metformin Standard stock solution accurately took 1 mL into 10 mL volumetric flask, and the volume was made up with the mobile phase (Figure 1).

Preparation of Standard Solution of Metformin Impurity A (25 ppm):

Standard stock solution of Metformin Impurity A was prepared by accurately adding 25mg of Metformin Impurity A into a 100ml volumetric flask and dissolved with methanol up to get 250µg/ml of Metformin Impurity A Standard Stock Solution. For the preparation of 25 µg/mL (25 ppm) of Metformin Impurity A Working standard solution. From the Metformin impurity, A Standard stock solution was accurately taken 1-mL

into a 10 mL volumetric flask, and the volume was made up with the mobile phase.

Preparation of Standard Solution of Teneligliptin (1 ppm)

Teneligliptin standard stock solution was prepared by accurately adding 10mg of Teneligliptin bulk drug into a 100 mL volumetric flask, which was dissolved in methanol up to mark obtain 100 µg/mL of Teneligliptin standard stock Solution. For the preparation of 10.0 µg/mL (10.0 ppm) of teneligliptin working standard stock solution (TWSSS). From the Teneligliptin Standard stock solution was accurately taken 1-mL into 10 mL volumetric flask, and final, the volume was made up with the mobile phase. For the preparation of 1.0 µg/mL (1.0 ppm) of TWSSS. From the Teneligliptin Standard stock solution was accurately taken 1ml into 10 mL volumetric flask, and finally, the volume was made up with the mobile phase (Figure 1).

Preparation of Standard Stock Solution of Teneligliptin Impurity B (10 ppm)

The standard stock solution of teneligliptin impurity B was prepared by accurately adding 10 mg of Teneligliptin Impurity B into a 100 mL volumetric flask and dissolving with methanol, and making up to the mark to obtain 100 µg/mL of Teneligliptin Impurity B solution. Accurately, Taken 1-mL into a 10-ml volumetric flask, and the final volume was made up with the methanol up to the mark to get 10 µg/mL Teneligliptin Impurity B Standard stock Solution. Teneligliptin Impurity B Standard stock solution was accurately taken 1-mL into 10 ml graduated flask, and the final volume was made up with the mobile phase to obtain 1.0 µg/mL (1.0 ppm) of teneligliptin impurity B working standard solution.

Preparation of Sample Solution from Pharmaceutical Marketed Tablets

About 10 tablets of Tenalifine M were weighed, and an average weight of 10 tablets was determined and powdered finely in a mortar. Powdered tablet equivalent to 10 mg of Teneligliptin and 250 mg of Metformin was accurately weighed and transferred into a 100 mL graduated flask and dissolved completely by sonicating method for 15 minutes with 60ml of mobile phase. After ensuring complete solubilization of drugs after final sonication volume was made up with mobile phase and filtered through 0.45-micron membrane filter, and then the filtrate is collected as a sample solution.

Chromatographic Separation

Standard solutions of Metformin and Teneligliptin, along with its related impurities, were injected 20 µL with a micro-syringe in a column. For appropriate minutes the chromatogram was run, and the detection was carried out at 225 nm wavelength. Chromatogram was stopped after separation was completely achieved. Recorded the data related to resolution, retention time, and peak like height, area, etc., by using the software.

Forced Degradation Study[31]

To evaluate the stability-indicating properties of the developed HPLC method, forced degradation studies were carried out following the ICH guidelines to produce the possible relevant degradants and test their chromatographic behavior.

Acid Degradation

Taken 1.0 mL of the stock solution into the 10 ml graduated flask and added 1.0 mL of 0.1 N HCl solution and mixed well with this solution it was kept for 5 hours at room temperature. After completing 5 hours, volume was adjusted with the mobile phase; after making final solution run into HPLC, the peak area and shape were observed under optimized chromatographic conditions.

Base Degradation

Taken 1.0 mL of stock solution, which transferred into the 10 ml volumetric flask, then added 1.0 ml of 0.1 N NaOH solution it mixed well, and it was kept for 5 hours at room temperature. After completing 5 hours, volume adjusted was with the mobile phase, after making the final solution run into HPLC and the peak area and shape observed under optimized chromatographic conditions.

Oxidative Degradation

1 mL of stock solution was taken and transferred into the 10 mL Erlenmeyer flask, and then added 1 mL of 3.0% H_2O_2 solution was mixed well and kept for 5 hours at room temperature. After completing 5 hours, volume was adjusted with the mobile phase. After making the final solution run into HPLC, the peak area and shape were observed under optimized chromatographic conditions.

Thermal Degradation

For the dry heat degradation study, the standard powder drugs were placed in an oven at 110ºC for 10 hours. Appropriate dilutions were prepared in the mobile phase and then analyzed under the optimized chromatographic conditions.

Photolytic Degradation

For the photo-degradation study, the standard powder drugs were exposed to UV light in a photo-stability chamber for 10 hours. Appropriate dilutions were prepared in mobile phase and then analyzed under the optimized chromatographic conditions.

Method Validation[31]

The RP-HPLC developed method was validated for impurities of Metformin and Teneligliptin as per ICH guidelines. The parameters were validated as accuracy, system suitability, system suitability, linearity, (inter-day precision, Intraday precision), LoD, LoQ, specificity, linearity and range, robustness, and system suitability.

System Suitability

The system suitability was calculated from different parameters like retention time, theoretical plates, resolution, and tailing factor.

Specificity

The developed RP-HPLC method of specificity was established by injecting 20 µL each of the working standard and sample solutions and blank solution..

Linearity

The linearity of a method is measured to see how well a calibration plot of response vs. concentration approximates a straight line. The linearity for metformin and its impurity and teneligliptin and its impurity were assessed by analysis of combined standard solution in a range of LoQ-37.50 µg/mL and LoQ-1.5µg/mL respectively. Suitable aliquots of the standard stock solutions of metformin and its impurity A (250 µg/mL) as well as teneligliptin and its impurity B (10 µg/mL) were transferred into a series of 10 mL volumetric flasks respectively to get concentration levels of LoQ, 50, 75,100, 125 and 150% of the respective standard concentration. The final volume was made up of diluents. Each mixed standard solution was injected and chromatograms were recorded. In terms of intercept, slope, correlation coefficient value, and the graph of peak area obtained versus respective concentration was plotted.

Precision

System precision was performed by injecting six replicates of a standard solution containing metformin and its impurity A (25.0 µg/mL) and teneligliptin and its impurity B (1.0 µg/mL) and chromatograms were recorded and areas of peaks were measured to calculate results of repeatability. A standard solution containing (LoQ, 25, 37.5 µg/mL) of metformin and its impurity A as well as standard solution containing (LoQ, 1.0, 1.5 µg/mL) of teneligliptin and its impurity B were assessed on different day for three times in interday precision and the same day in intraday precision and %RSD was calculated.

Accuracy

To check the accuracy of the proposed method for determination of metformin impurity A and for teneligliptin impurity B, recovery studies were carried out at LoQ, 80, 100 and 120% of the test concentration according to ICH guidelines. The recovery study was performed three times at each level.

LoD and LoQ

LoD and LoQ for both the drugs and their respective impurities were estimated using the linearity data. Then LoQ and LoD were calculated with formula given below.

$$LoQ=10 * SD/slope\ of\ calibration\ curve$$

$$LoD= 3.3 * SD/slope\ of\ calibration\ curve$$

Robustness

The robustness study was carried out in the chromatographic conditions to evaluate the influence of small but deliberate variations, which have been described in the Chromatographic conditions section. The factors chosen for this study, which were critical sources of variability in the operating procedures, such as the ratio of mobile phase was changed ±2mL, and flow rate of mobile phase was changed ±0.2 mL/min, were identified. In all these experiments, the mobile-phase components were not changed and their effect was observed on system suitability for standard preparation.

Known and Unknown Impurities of Metformin and Teneligliptin

Analyzed test solution three times and calculated % of each known and unknown impurities compared with standard preparations of metformin and teneligliptin. The amount of known and unknown related impurities present in the formulation of metformin and teneligliptin was calculated.

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

Chromatographic parameters were preliminary optimized to develop a stability-indicating Related Substances method for Metformin and Teneligliptin. Metformin and Teneligliptin have one impurity each. So these impurities need to separate from each other and the main analyte to show the stability-indicating Related Substances method. The method development process was carried out by examining different conditions like mobile phase compositions like Water: Methanol, Water: Acetonitrile, buffer: methanol, Acetonitrile: buffer with different ratios. Metformin, teneligliptin, metformin impurity A and teneligliptin impurity B were found to show a significant UV absorbance at 225 nm, so this wavelength was chosen for UV detection. By use of a C18 column, it was found that the mobile phase consisting of Buffer [Buffer (0.05 M KH_2PO_4, pH 4.0): Acetonitrile (80:20) provided a well-defined peak shape with good resolution. The peaks with retention time (RT) of 5.280 minutes and 7.460 minutes for metformin and

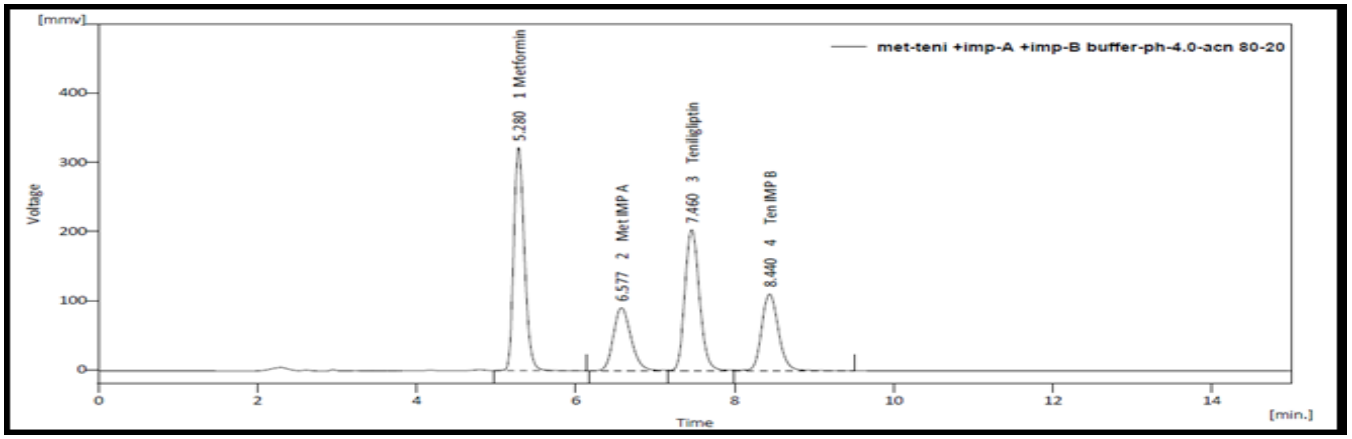

Figure 2: Optimized chromatogram of metformin, teneligliptin, metformin impurity A, and teneligliptin impurity B in buffer (pH 4.0): acetonitrile (80:20v/v)

teneligliptin and the retention time of Metformin impurity A and Teneligliptin impurity B were found to be 6.577 minutes and 8.440 minutes, respectively (Figure 2). The representative chromatograms (Figure 2) which shows a significant amount of resolution and good peak shapes with the selected mobile phase.

The final optimized chromatographic condition for Metformin, Metformin Impurity A, Teneligliptin and Teneligliptin Impurity B having Stationary phase used BDS Hypersil C18 (250 mm×4.6 mm, 5 µm particle size), mobile phase used buffer (pH 4.0): Acetonitrile (20:80 v/v), used detection wavelength was 225 nm, with flow rate was 1 mL/min with injection volume is 20 µL. For run time 15 minutes

Forced Degradation Studies

The sample was injected under various stress conditions. The acidic degradation, base degradation, oxidative degradation, thermal degradation and photodegradation were performed as per procedure, and %degradation was calculated from the chromatographic peaks. Metformin and teneligliptin in standard as well as sample mixture in acid degradation, base degradation, oxidative degradation, thermal degradation and photo degradation. The details of %degradation are given in the Table 1. However, Metformin and Teneligliptin standard and sample did not show thermal as well as photo degradation.

Method Validation

The proposed method was validated with accuracy, precision, linearity, specificity, system suitability, LoD and LoQ, and robustness.

System Suitability

The System Suitability was calculated from different parameters like retention time, theoretical plates, resolution, and tailing factor. System suitability was used to verify the repeatability and resolution of the system were sufficient for the analysis intended. The system suitability parameters observed for metformin have a retention time of 5.280, theoretical plates per column of 7180, and tailing factor of 1.380. The system suitability parameters observed for metformin impurity A have a retention time of 6.577, theoretical plates per column 4160, and tailing factor 1.310. The system suitability parameters observed for teneligliptin have a retention time of 7.460, theoretical plates per column 7708, and tailing factor of 1.245. The system suitability parameters observed for Teneligliptin Impurity B have a retention time of 8.440, theoretical plates per column 7681, and tailing factor of 1.232.

Specificity

The method's specificity was established by studying the resolution factor of drug peaks from the nearest resolving peak and among all other peaks. The specificity of the chromatographic method was determined to ensure the separation of metformin, teneligliptin, metformin impurity A and teneligliptin impurity B. The chromatograms of metformin and teneligliptin along with its related impurity of metformin and teneligliptin sample and standards show no interference with the chromatogram of teneligliptin and metformin blank, so the developed method is specific Figure 3.

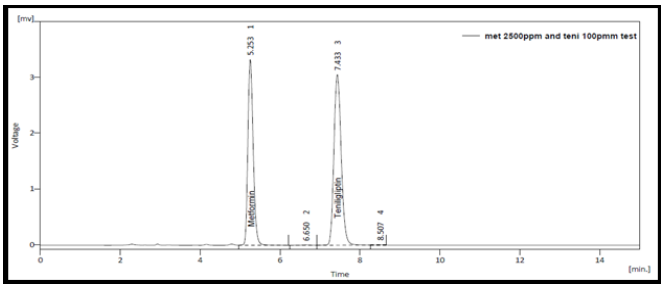

Figure 3: Chromatogram of Metformin and Teneligliptin Sample

Table 1: Results of forced degradation

Parameter (Degradation)	*Metformin*				*Teneligliptin*			
	Standard		*Sample*		*Standard*		*Sample*	
	Area	*% Degradation*	*Area*	*% Degradation*	*Area*	*% Degradation*	*Area*	*% Degradation*
Acid	24798.412	17.91 %	25516.48	15.54%	33990.220	14.247%	33528.788	15.411%
Base	25708.117	14.90%	25213.76	16.54%	32493.498	18.023%	33063.754	16.584%
Oxidative	24357.846	19.37%	24675.36	18.32%	33669.814	15.055%	33184.230	16.280%
Thermal	30323	-	30322.99	-	40045.44	-	40288.87	-
Photo	31203.01	-	31459.05	-	40234.38	-	39890.89	-

Table 2: Linearity data for metformin, teneligliptin, and its related impurities

Sr. No.	*Linearity Level*	*Concentration(µg/mL)*		*Area*		*Concentration(µg/mL)*		*Area*	
		Metformin	*Metformin Imp A*	*Metformin*	*Metformin Imp A*	*Teneligliptin*	*Teneligliptin Imp B*	*Teneligliptin*	*Teneligliptin Imp B*
1	LOQ	2.5	0.62	41.117	31.761	0.1	0.1	59.667	23.784
2	50%	12.5	12.5	122.982	104.375	0.5	0.5	198.885	78.995
3	75%	18.75	18.75	179.267	155.239	0.75	0.75	289.558	117.741
4	100%	25	25	245.324	207.427	1	1	396.117	157.557
5	125%	31.25	31.25	302.072	258.03	1.25	1.25	487.404	196.245
6	150%	37.5	37.5	366.765	366.765	1.5	1.5	591.841	236.321

Linearity

The linearity for Metformin and its Related Impurity A and Teneligliptin and its Related impurity B were assessed by analysis of combined standard solution in a range of LoQ-37.50µg/mL and LoQ-1.5µg/mL, respectively. The correlation coefficient for the calibration curve of Metformin and its Related Impurity A and Teneligliptin and its related impurity B was found to be NLT 0.999, respectively (Table 2).

Precision

Repeatability

The repeatability data of peak area measurement for Metformin and its Related Impurity A and Teneligliptin and its Related Impurity B, based on six measurements of same solution. The mean area observed 243.497 for Metformin and 205.805 for Metformin impurity A at concentrations 25 µg/mL with % RSD 1.480, and 1.717, respectively while the mean area observed 391.827 for Teneligliptin and 156.595 for Teneligliptin impurity B at a concentration of 1.0 µg/mL with %RSD 1.617 & 1.514 respectively. The repeatability shows that the % RSD values observed within the acceptance limit of NMT 5% (Table 3).

Intraday Precision and Inter Day Precision

The data for intraday precision as well as inter-day precision for metformin and its related impurity A and teneligliptin and its related impurity B is shown in Table 3. The %RSD calculated and all values are within the acceptance limit. Hence the method is précised.

Accuracy

To check the accuracy of the proposed method for determination of metformin impurity A and for teneligliptin impurity B, recovery studies were carried out at LoQ, 80, 100, and 120% of the test concentration according to ICH guidelines. The method accuracy was established by a recovery study from marketed formulation at three levels of standard addition. The percentage recovery for metformin impurity A was 96.181 to 102.816%, and the Percentage recovery for Teneligliptin Impurity B was 96.999 to 103.824% (Table 4).

LoD and LoQ

Limit of detection and limit of quantitation for both the drugs and their respective impurities were estimated using the linearity data (Figure 5). Repeated calibration

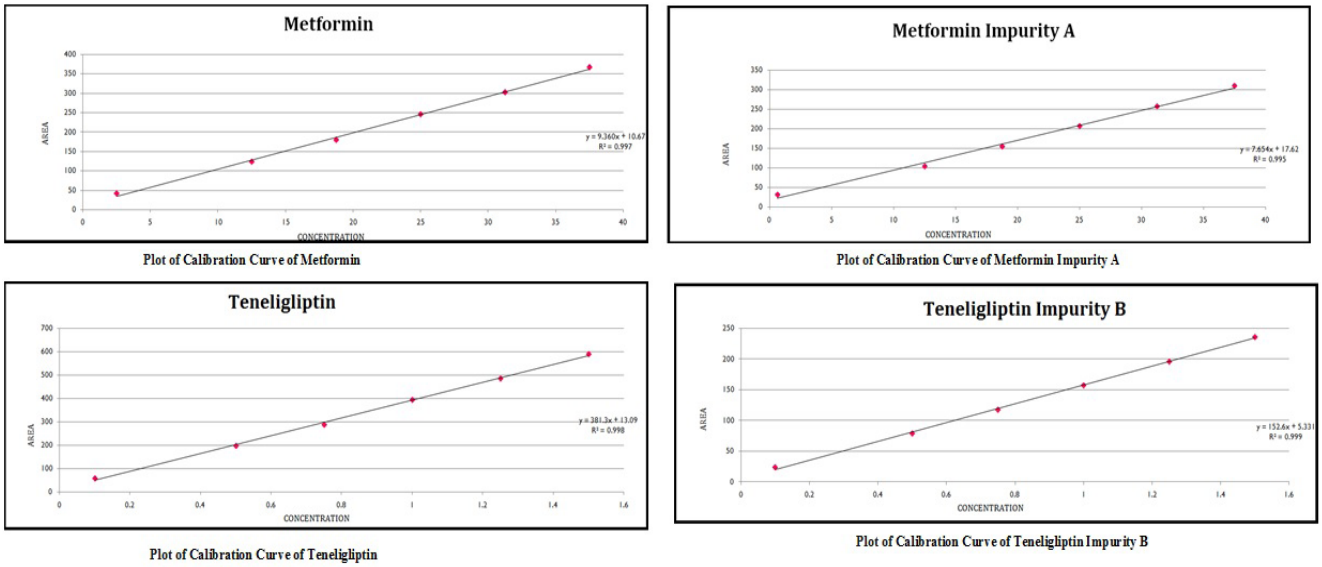

Figure 4: Plots of calibration curves of metformin, teneligliptin and related impurities

Table 3: Intraday precision and inter day precision data for estimation of metformin, teneligliptin and its related impurities

Conc. (µg/mL)	*Area Mean ± S.D. (n = 3)*	*%R.S.D*	*Area Mean ± S.D. (n = 3)*	*% R.S.D*	*Conc. (µg/mL)*	*Area Mean ± S.D. (n = 3)*	*%R.S.D*	*Area Mean ± S.D. (n = 3)*	*%R.S.D*
Metformin			*Metformin impurity A*		*Teneligliptin*			*Teneligliptin impurity B*	
Intraday precision									
LOQ	40.335 ± 1.097	2.719	31.414 ± 0.904	2.877	LoQ	67.920 ± 2.277	3.352	23.218 ± 1.018	4.385
25	245.411 ± 3.920	1.597	205.114 ± 5.963	2.907	1.0	395.859 ± 5.635	1.424	156.267 ± 3.851	2.465
37.5	361.530 ± 3.826	1.058	307.101 ± 4.859	1.582	1.5	582.476 ±7.598	1.304	232.365 ± 5.715	2.460
Inter day Precision									
LOQ	41.222 ± 1.041	2.525	31.573 ± 0.770	2.438	LoQ	68.552 ± 0.937	1.371	22.868 ± 0.346	1.513
25	241.331 ± 1.950	0.808	205.572 ± 3.203	1.558	1.0	385.008 ± 5.448	1.415	155.154 ± 1.198	0.772
37.5	365.423 ± 6.980	1.910	308.333 ± 4.181	1.356	1.5	586.731 ± 6.320	1.077	232.884 ± 1.425	0.615

Table 4: Recovery data for metformin impurity A and teneligliptin impurity B

Sr. No.	*Conc. level (%)*	*Area of recovery spiked with Test*	*Area of impurity in test*	*Net area of Std*	*Area of Std*	*Amount added (µg/ml)*	*Amount recovered (µg/mL)*	*%Recovery*	*%RSD*
Metformin Impurity A									
1		36.994	34.587	2.407	104.274	0.6	0.577	96.181	
2	LoQ	37.095	34.587	2.508	104.274	0.6	0.601	100.217	2.072
3		37.057	34.587	2.47	104.274	0.6	0.592	98.698	
4		118.337	34.587	83.750	104.274	20	20.079	100.397	
5	80%	116.473	34.587	81.886	104.274	20	19.632	98.162	1.656
6		119.171	34.587	84.584	104.274	20	20.279	101.396	
7		137.802	34.587	103.215	104.274	25	24.746	98.984	
8	100%	141.663	34.587	107.076	104.274	25	25.672	102.687	1.845
9		140.092	34.587	105.505	104.274	25	25.295	101.181	
10		158.918	34.587	124.331	104.274	30	29.809	99.362	
11	120%	162.092	34.587	127.505	104.274	30	30.570	101.899	1.765
12		163.239	34.587	128.652	104.274	30	30.845	102.816	
Teneligliptin impurity B									
1		63.054	55.398	7.656	78.929	0.1	0.097	96.999	
2	LoQ	63.524	55.398	8.126	78.929	0.1	0.103	102.953	3.057
3		63.202	55.398	7.804	78.929	0.1	0.099	98.874	
4		119.512	55.398	64.114	78.929	0.8	0.812	101.537	
5	80%	120.956	55.398	65.558	78.929	0.8	0.831	103.824	2.941
6		117.232	55.398	61.834	78.929	0.8	0.783	97.927	
7		136.649	55.398	81.251	78.929	1	1.029	102.942	
8	100%	133.343	55.398	77.945	78.929	1	0.988	98.753	2.804
9		132.460	55.398	77.062	78.929	1	0.976	97.635	
10		151.703	55.398	96.305	78.929	1.2	1.220	101.679	
11	120%	149.143	55.398	93.745	78.929	1.2	1.188	98.976	1.354
12		150.210	55.398	94.812	78.929	1.2	1.201	100.103	

curve 5 times and calculated standard deviation of the intercepts. The limit of detection for metformin was observed 0.940 µg/mL, for metformin impurity A observed 0.206 µg/mL, for teneligliptin observed 0.038 µg/mL and for teneligliptin impurity B observed at 0.009 µg/mL. However, the LoQ for metformin observed 2.849 µg/mL, for metformin impurity A observed 0.623 µg/mL, for teneligliptin observed 0.116 µg/mL and for teneligliptin impurity B observed 0.026 µg/mL.

Robustness

The robustness study was carried out to assessed the influence of small but deliberate variations in the chromatographic conditions. The chromatographic factors as ratio of Mobile phase was changed ±2mL and flow rate of mobile phase ± 0.2 mL/min, was changed without changing the mobile phase components and their effect observed on system suitability for standard preparation. The results show that the changing effect was found to be within the acceptance criteria and the % RSD values were observed within the standard limit of not more than 5%.

Known and Unknown Impurities of Metformin and Teneligliptin

The proposed method's appropriateness was tested by analyzing the commercially available Tablet formulation Tenalifine M. The results of known and unknown impurities are calculated in %RSD. The %RSD for known impurity, Metformin impurity A observed 4.513% while Teneligliptin impurity B was observed at 3.289%. However, the % RSD for a single unknown impurity was observed at 2.148% the total unknown impurity was observed 1.928%. The % RSD values were observed within the standard limit of not more than 5%.

The results indicate that the developed method is accurate, simple, precise, and rapid. It can be used in the regular quality control of dosage forms in industries.

RP-HPLC method was validated according to ICH guidelines. And it was found to be linear within the range Correlation co-efficient for calibration curve of Metformin, and its Related Impurity A and Teneligliptin and its related impurity B was found to be NLT 0.999 respectively. The accuracy of the method was determined at 80, 100, and 120%

Table 5: Robustness data for Metformin, Teneligliptin, and its related impurities

Sr. No.	*Area at Flow rate (+0.2 ml/min)*	*Area at Flow rate (-0.2 ml/min)*	*Area at pH (+0.2)*	*Area at pH (-0.2)*	*Area at Mobile phase (+2)*	*Area at Mobile phase(-2)*
Metformin						
1	234.099	255.199	232.644	253.564	231.945	252.799
2	235.041	257.001	230.685	252.001	232.877	249.063
3	232.613	251.069	229.597	255.199	229.194	254.584
%RSD	0.523	1.195	0.669	0.631	0.828	1.117
Metformin impurity A						
1	195.886	214.404	196.675	217.368	206.068	232.917
2	192.571	210.737	204.909	223.738	212.985	248.486
3	191.114	218.726	208.135	218.027	220.086	237.977
%RSD	1.266	1.863	2.908	1.594	3.290	3.312
Teneligliptin						
1	378.033	412.128	375.615	409.466	374.486	408.23
2	379.554	415.039	369.723	410.31	375.991	403.257
3	370.822	408.805	371.205	397.481	369.537	410.794
%RSD	1.240	0.757	0.823	1.768	0.904	0.941
Teneligliptin impurity B						
1	148.879	162.774	149.479	165.029	156.653	176.889
2	143.959	161.239	156.817	173.661	161.927	186.036
3	145.856	166.05	159.497	172.19	165.643	180.069
%RSD	1.697	1.504	3.340	2.712	2.799	2.566

levels. The percentage recovery for metformin impurity A was 96.181–102.816%, and the percentage recovery for teneligliptin impurity B was 96.999–103.824%. The LoD for metformin was found to be 0.940 µg/mL, and for metformin impurity A was 0.206 while for teneligliptin was 0.038 µg/mL and for teneligliptin impurity B was 0.009 µg/mL. Also, the LoQ of metformin was found to be 2.849 µg/mL, and for metformin impurity A was 0.623 µg/mL and for teneligliptin 0.116 µg/mL, and for teneligliptin impurity B was 0.026 µg/mL indicating the sensitivity of the method (Table 5). The method developed was found to be precise as the %RSD values for intra-day and inter-day were found to be less than 5.0%. And the method was also found to be robust, indicated by the % RSD values of less than 5%.

CONCLUSION

There is no analytical work has been available regarding the related Impurities RP-HPLC method for Metformin and Teneligliptin in the literature. Data regarding the behavior of the drug and its related impurities in chromatographic conditions and other relevant analytical properties are not available. It is a novel attempt in a field of research that has been made to validate and develop the Related Impurities method via RP- HPLC. Conclusively, the RP HPLC method described in this paper is specific, sensitive, rapid, and easy to perform. The proposed RP-HPLC method enables the simultaneous estimation of metformin and teneligliptin and its related impurities. This method provides good separation and resolution of the chromatographic peaks of the metformin, teneligliptin, and its related impurities. The 0.05 M Potassium Dihydrogen Phosphate (pH 4.0): Acetonitrile (80:20v/v) was used as the mobile phase. The sample recoveries from all formulations agreed with their respective label claims, which suggested non-interference of formulations excipients in the estimation. The method was successfully validated in terms of specificity, precision, linearity, accuracy, and robustness as per ICH guidelines. It can be concluded that the proposed method can be used for routine analysis for estimation of related impurities of Metformin and Teneligliptin in combined dosage form by RP-HPLC.

ABBREVIATIONS

RP-HPLC: Reverse Phase High Performance Liquid Chromatography; mm: millimetre; M: Molar; µm: Micrometer; pH: Potential of Hydrogen; ml: Milliliters; nm: nanometer; %: Percentage; v/v: Volume/volume; LOD: Limit of detection; LOQ: Limit of quantitation; ICH: International Conference on Harmonization; µg: microgram; % RSD: Relative standard deviation; NMT: Not more than; NLT: Not less than; ISD: Standard deviation; Rs: Resolution; °C: Degree Celsius; µg: Microgram;; mg: Milligrams; min: Minutes; MET: Metformin; AMPK: Adenosine monophosphate-activated protein kinase; UV: Ultra violet; pvt: private; g: gram; ppm: parts per million; fig: figure.

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