

Development and Validation of Analytical Method for Simultaneous Estimation of Teneligliptin, Metformin and Pioglitazone in Pharmaceutical Dosage Form

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ABSTRACT

Objective: To develop a simple, precise, accurate, method was developed and validated for analysis of Teneligliptin, Metformin and Pioglitazone in pharmaceutical dosage form.

Method: The adequate separation was carried out by using Waters UPLC with Welch C18 (150×4.6mm, 2 μm) column with the mobile phase composed of Methanol: ACN: Phosphate Buffer pH 2.5 (70:05:25) and the pH was adjusted using orthophosphoric acid. The Flow rate was set at 0.5 ml/min and a detection wavelength of 238 nm using a photodiode array detector.

Result: The complete analytical method validation was successfully carried out as per ICH guidelines. The retrieval study was carried out at 50% to 150% level of working concentration, and results were in the range of 98 to 102%. The linearity of Teneligliptin, Metformin and Pioglitazone were in the range of 0.08-4 μg/ml, 2-100 μg/ml and 0.06-3 μg/ml respectively. The linear regression curve ($R^2 = 0.999$) with limits of detection (LOD) and quantitation (LOQ) being 0.04 and 0.12 μg/ml for Teneligliptin, 0.32 and 0.97 μg/ml for Metformin and 0.19 and 0.58 μg/ml for Pioglitazone respectively. The retention time for Teneligliptin was 3.00 min, for Metformin was 2.55 min and for

Pioglitazone was 3.92 min. The method shows good recoveries and intra-day and inter-day relative standard deviations were less than 2%. Validation parameters as ruggedness and robustness were also determined as per ICH guidelines and were found to be satisfactory.

Conclusion: The developed UPLC method can be successfully used for the simultaneous estimation of Teneligliptin, Metformin and Pioglitazone in pharmaceutical dosage form.

Key Words: Teneligliptin, Metformin, Pioglitazone, UPLC, Validation.

I. INTRODUCTION

Teneligliptin IUPAC name {(2S,4S)-4-[4-(5-Methyl-2-phenylpyrazol-3-yl)piperazin-1-yl]pyrrolidin-2-yl}-(1,3-thiazolidin-3-yl)methanone. Chemical formula $C_{22}H_{30}N_6OS$ (Fig.1). It is an anti-diabetic drug in the Dipeptidase-4 (DPP-4) inhibitors class of drugs. The Glucagon increased blood glucose levels, and DPP-4 inhibitors reduce glucagon and blood glucose levels. The mechanism of DPP-4 inhibitors is to increase incretin levels which inhibit glucagon release, which in turn increases insulin secretion, decrease gastric emptying, and decrease blood glucose levels.

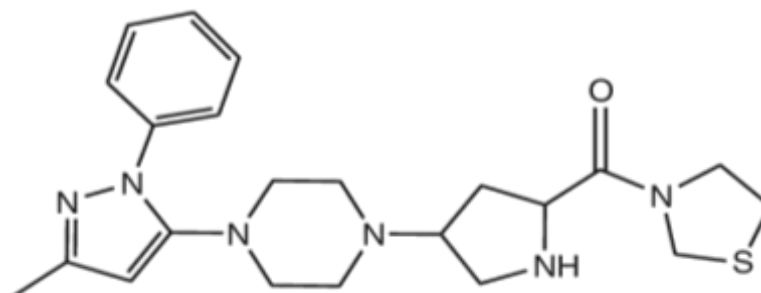


Fig.1 Chemical structure of Teneligliptin

Metformin IUPAC name N, N-Dimethylimidodicarbonimidic diamide. Chemical formula $C_4H_{11}N_5$ (Fig.2). It is anti-diabetic drug in the Biguanide category. It decreases blood glucose

levels by decreasing hepatic glucose production (also called gluconeogenesis), decreasing the intestinal absorption of glucose, and increasing insulin sensitivity by increasing peripheral glucose

uptake and utilization. It is well established that Metformin inhibits mitochondrial complex I activity, and it has since been generally postulated that its potent antidiabetic effects occur through

this mechanism. The above processes lead to a decrease in blood glucose, managing type II diabetes and exerting positive effects on glycaemic control.

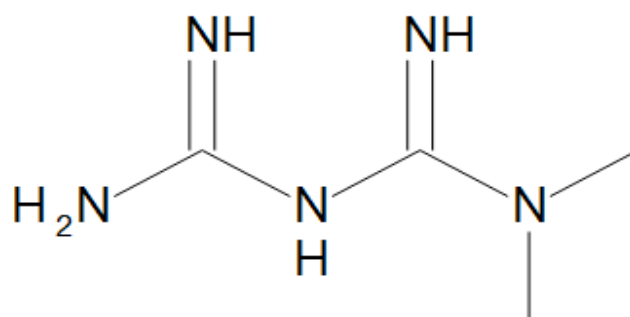


Fig.2 Chemical structure of Metformin

Pioglitazone IUPAC name 5-(4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl)thiazolidine-2,4-dione. Chemical formula C₁₉H₂₀N₂O₃S (Fig.3). It is an anti-diabetic drug in the thiazolidinedione class of drugs. Pioglitazone is a selective agonist at peroxisome proliferator-activated receptor-gamma (PPAR γ) in target tissues for insulin action such as adipose tissue, skeletal muscle, and liver. Activation of PPAR γ increases the

transcription of insulin-responsive genes involved in the control of glucose and lipid production, transport, and utilization. Through this mechanism, pioglitazone both enhances tissue sensitivity to insulin and reduces the hepatic production of glucose (i.e. gluconeogenesis) - insulin resistance associated with type 2 diabetes mellitus is therefore improved without an increase in insulin secretion by pancreatic beta cells.

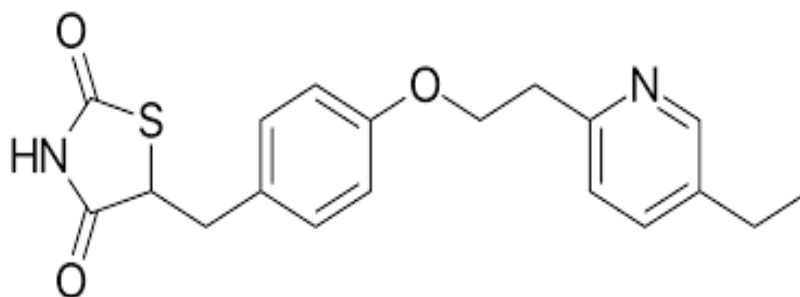


Fig.3 Chemical structure of Pioglitazone

Methods

Reagents and chemicals

All the Chemicals and Solvents used were of analytical grade used of (RANKEM, INDIA). Solvents and solutions were filtered through a membrane filter (0.45 μ m pore size) and degassed by sonication before use.

Instrumentation

The chromatographic analysis was performed on Waters Alliance UPLC system equipped with PDA detector. The output signals were monitored and processed using LC Solution software. The analytical column was Welch C18 (4.6 mm \times 150 mm, 2 μ m) and the samples were

introduced through a injection valve with 5 μ l sample loop.

Wavelength Detection

25mg of Teneligliptin, 25mg of Metformin & 25mg Pioglitazone take into 25ml volumetric flask separately and dissolved with diluent (stock-1 solution) (Teneligliptin 1000 μ g/ml, Metformin 1000 μ g/ml and Pioglitazone 1000 μ g/ml). From that 1ml in 10ml volumetric flask separately (stock-2 solution) (Teneligliptin 100 μ g/ml, Metformin 100 μ g/ml and Pioglitazone 100 μ g/ml). From that 1ml in 10ml volumetric flask separately (Working standard solution) (Teneligliptin 10 μ g/ml, Metformin 10 μ g/ml and

Pioglitazone 10 μ g/ml). UV Spectra was taken between range of 200-400nm using UV-Visible Double beam spectrometer. Absorbance of all three Teneiglipitin, Metformin and Pioglitazone was observed at 246nm, 236nm and 268nm respectively.

Chromatographic conditions

Mobile phase selection involved selection of solvent, selection of buffer, pH of buffer and ratio of buffer and solvent. The standard solutions of Teneiglipitin, Metformin and Pioglitazone were injected into the UPLC system and run in different solvent system. Various ratios of mobile phase containing Methanol: Water, ACN: Water, Phosphate Buffer pH 4.0: Methanol, Phosphate Buffer pH 6.0: Methanol were tried in order to find the best conditions for the separation of all three drugs. It was found that Methanol, CAN and Phosphate buffer pH 2.5 gives satisfactory result. Finally, Methanol: ACN: Potassium Dihydrogen Phosphate buffer pH 2.5 (70:05:25 %v/v/v) ratio was optimized as the mobile phase for the determination. pH was set by using 1% orthophosphoric acid. Injection volume was 5 μ l, Flow rate was 0.5 ml/min and the eluent was detected at 238nm at column temperature 25°C. These conditions showed sharp peak of Teneiglipitin, Metformin and Pioglitazone with retention time of 3.00min, 2.55min and 2.92min respectively.

Preparation of Standard solution and Sample solution

Standard solution: Weigh 10mg of Teneiglipitin, 10mg of Metformin and 10mg of Pioglitazone. Transferred into 3 different 100ml of volumetric flask and volume was made upto mark with diluent. [Standard stock-1 solution of Teneiglipitin (100 μ g/ml), Metformin (100 μ g/ml) and Pioglitazone (100 μ g/ml)]. Further Dilution. From stock-1 solution of Teneiglipitin and Pioglitazone pipetted out 0.8ml and 0.6ml in separate 10ml volumetric flask and dilute to the mark with diluent [Standard stock-2 solution of Teneiglipitin (8 μ g/ml) and Pioglitazone (6 μ g/ml)]. Further Dilution. For Teneiglipitin pipetted out 2ml from stock-2 solution, Metformin pipetted out 4ml from stock-1 solution and for Pioglitazone pipetted out 2ml from stock-2 solution into 10ml volumetric flask and dilute to the mark with diluent (working standard of Teneiglipitin 1.6 μ g/ml, Metformin 40 μ g/ml, and Pioglitazone 1.2 μ g/ml).

Sample solution: (Label claim: Teneiglipitin - 20mg, Metformin- 500mg and Pioglitazone - 15mg)

Twenty tablets were weighed; average weight was calculated and tablets were powdered finely. Tablet Powder equivalent to 2mg of Teneiglipitin, 50mg of Metformin and 1.5mg of Pioglitazone were added into 50ml of volumetric flask. Teneiglipitin (400 μ g/ml), Metformin (1000 μ g/ml) and Pioglitazone (300 μ g/ml). Volume was made up to the mark with diluent. 1ml of above solution was transferred to 10ml volumetric flask. Volume was made up to the mark with diluent, which gives Teneiglipitin (4 μ g/ml), Metformin (100 μ g/ml) and Pioglitazone (3 μ g/ml). 4ml of above solution was transferred to 10ml volumetric flask. Volume was made up to the mark with diluent, which gives Teneiglipitin (1.6 μ g/ml), Metformin (40 μ g/ml) and Pioglitazone (1.2 μ g/ml). The quantification was carried out by keeping these values to be straight line equation of calibration curve.

Analytical method validation

1. Specificity:

Demonstration of specificity is required to show that the procedure is unaffected by the presence of impurities or excipients. Specificity of an analytical method indicates that the analytical method is its able to measure accurately and specifically the analyte of interest without any interference from blank. So here, the specificity was determined by the comparison of the chromatograms of

- Blank (mobile phase).
- Standard solutions Teneiglipitin, Metformin and Pioglitazone.
- Sample solution of Teneiglipitin, Metformin and Pioglitazone.

2. Linearity:

The linearity for Metformin, Teneiglipitin and Pioglitazone was assessed by analysis of standard solution in range of 2-100 μ g/ml for Metformin, 0.08-4 μ g/ml for Teneiglipitin and 0.06-3 μ g/ml for Pioglitazone.

To obtain 2-100 μ g/ml of Metformin solution make 3 stock solution. 500 μ g/ml (25mg powder in 50ml flask), 100 μ g/ml (2ml from 500 μ g/ml in 10ml flask) and 10 μ g/ml (1ml from 100 μ g/ml in 10ml flask) and makeup with methanol upto mark in each flask and labeled as stock-1,2 & 3 solution respectively. To obtain 0.08-4 μ g/ml of Teneiglipitin solution make 4 stock solution. 100 μ g/ml (10mg powder in 100ml flask),

80µg/ml (8ml from 100µg/ml in 10ml flask), 8µg/ml (1ml from 80µg/ml in 10ml flask) and 0.8µg/ml (1ml from 8µg/ml in 10ml flask) and makeup with methanol upto mark in each flask and labeled as stock-1,2,3 & 4 solution respectively. To obtain 0.06-3µg/ml of Pioglitazone solution make 4 stock solution. 100µg/ml (10mg powder in 100ml flask), 60µg/ml (6ml from 100µg/ml in 10ml flask), 6µg/ml (1ml from 60µg/ml in 10ml flask) and 0.6µg/ml (1ml from 6µg/ml in 10ml flask) and makeup with methanol upto mark in each flask and labeled as stock-1,2,3 & 4 solution respectively.

To obtain 100,80,60 & 40µg/ml for Metformin pipetted out 2,1.6,1.2 & 0.8ml from stock-1 solution of Metformin in each 10ml volumetric flask and labeled as working solution 1,2,3 & 4 respectively. To obtain 4,3,2,2.4 & 1.6µg/ml for Teneiglipitin pipetted out 0.8,0.6,0.4 & 0.2ml from stock-2 solution of Teneiglipitin in previous flask which is labeled working solution 1,2,3 & 4 respectively. To obtain 3,2.4,1.8 & 1.2µg/ml for Pioglitazone pipetted out 0.8,0.6,0.4 & 0.2ml from stock-2 solution of Pioglitazone in previous flask which is labeled working solution 1,2,3 & 4 respectively and makeup with methanol upto mark.

To obtain 20 & 10µg/ml for Metformin pipetted out 2 & 1ml from stock-2 solution of Metformin in each 10ml volumetric flask and labeled as working solution 5 & 6 respectively. To obtain 0.8 & 0.4µg/ml for Teneiglipitin pipetted out 1 & 0.5ml from stock-3 solution of Teneiglipitin in previous flask which is labeled working solution 5 & 6 respectively. To obtain 0.6 & 0.3µg/ml for Pioglitazone pipetted out 1 & 0.5ml from stock-3 solution of Pioglitazone in previous flask which is labeled working solution 5 & 6 respectively and makeup with methanol upto mark.

To obtain 2µg/ml for Metformin pipetted out 2ml from stock-3 solution of Metformin in 10ml volumetric flask and labeled as working solution 7. To obtain 0.08µg/ml for Teneiglipitin pipetted out 1ml from stock-4 solution of Teneiglipitin in previous flask which is labeled working solution 7. To obtain 0.06µg/ml for Pioglitazone pipetted out 1ml from stock-4 solution of Pioglitazone in previous flask which is labeled working solution 7 and makeup with methanol upto mark.

In term of slope, intercept and correlation coefficient value, the graph of peak area obtained versus respective concentration was plotted. (Fig. 5).

Acceptance criteria: value of r^2 should be nearer to 1 or 0.9999.

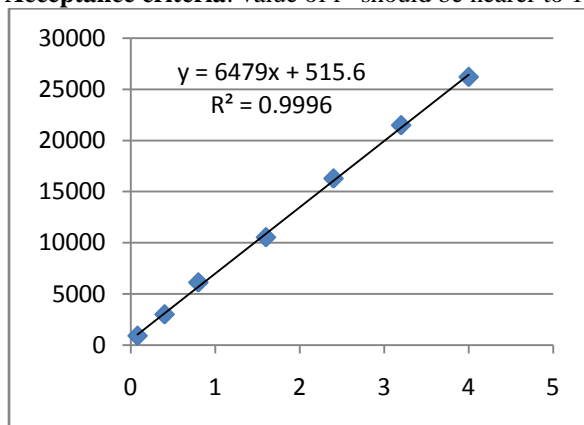


Fig.4 Calibration curve of Teneiglipitin (1.6 µg/ml)

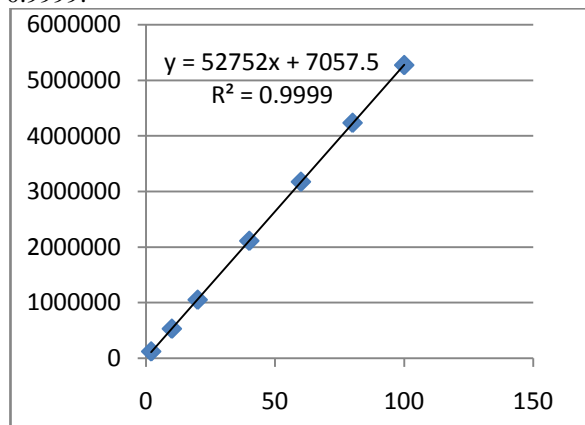


Fig.5 Calibration curve of Metformin (40 µg/ml)

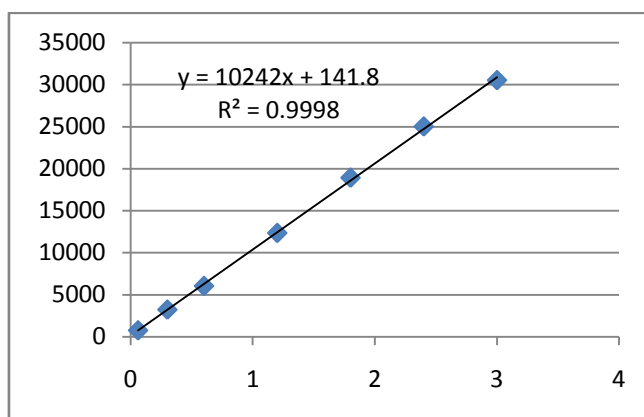


Fig.6 Calibration curve of Pioglitazone (1.2 µg/ml)

3. Precision:

Precision can be performed at two different levels: repeatability and intermediate precision. Repeatability refers to the use of the analytical procedure within the laboratory over the shorter period of the time that was evaluated by assaying the samples during the same day. Repeatability was carried out using six replicates of the sample injection. Intra-day precision was determined by analyzing, the three different concentrations for three times in the same day. Day to day variability was assessed using above mentioned three concentrations analyzed on three consecutive days for inter-day precision. Results should be expressed as Relative standard deviation (RSD) or co-efficient of variance.

A. Repeatability:

Standard solution containing Teneligliptin, Metformin and Pioglitazone (1.6, 40 and 1.2µg/ml respectively) was injected six times and areas of peaks were Measured and RSD was calculated.

B. Interday Precision:

Standard solution containing Teneligliptin (0.8, 1.6, 2.4µg/ml), Metformin (20, 40, 60µg/ml) and Pioglitazone (0.6, 1.2, 1.8µg/ml) respectively were injected three times in same day and areas of peaks were measured and RSD was calculated.

C. Intraday Precision:

Standard solution containing Teneligliptin (0.8, 1.6, 2.4µg/ml), Metformin (20, 40, 60µg/ml) and Pioglitazone (0.6, 1.2, 1.8µg/ml) were injected three times in different days and areas of peaks were measured and RSD was calculated.

Acceptance criteria: RSD of area should not be more than 2.0%.

4. Accuracy:

Preparation of Standard Stock Solution of Stock-1 Solution of Teneligliptin, Metformin and Pioglitazone:

Accurately weighed Teneligliptin (10mg) was transferred into 100ml of volumetric flask and make upto the mark with diluent (Teneligliptin 100µg/ml). Accurately weighed Metformin (10mg) was transferred into 100ml of volumetric flask and make upto the mark with diluent (Metformin 100µg/ml). Accurately weighed Pioglitazone (10mg) was transferred into 100ml of volumetric flask and make upto the mark with diluent (Pioglitazone 100µg/ml).

Preparation of Standard Stock-2 Solution of Teneligliptin, Metformin and Pioglitazone:

From the stock-1 solution of Teneligliptin and Pioglitazone pipetted out 0.8ml and 0.6ml in separate 10ml volumetric flask and dilute to the mark with diluent [Standard stock-2 solution of Teneligliptin (8µg/ml) and Pioglitazone (6µg/ml)].

Preparation of Working Standard Solution of Metformin, Teneligliptin, Metformin and Pioglitazone:

For Teneligliptin pipetted out 2ml from stock-2 solution, for Metformin pipetted out 4ml from stock-1 solution, and for Pioglitazone pipetted out 2ml from stock-2 solution into 10ml volumetric flask and dilute to the mark with diluent(working standard solution of Teneligliptin 1.6µg/ml, Metformin 40µg/ml and Pioglitazone 1.2µg/ml).

Preparation of Sample for Recovery:

Teneligliptin, Metformin and Pioglitazone (1.6, 40 and 1.2µg/ml respectively) drug solution was taken in three different flask labeled A, B and C. Spiked 50%, 100%, 150% of working standard

solution in it and diluted up to 10ml. The area of each solution peak was measured.

The amount of Teneligliptin, Metformin, and Pioglitazone was calculated at each level and % recoveries were calculated.

5. Limit of detection (LOD) and limit of quantitation (LOQ)

Sensitivity of the proposed method was estimated in terms of LOD and LOQ. LOD is the lowest concentration in a sample that can be detected, but not necessarily quantified; under the stated experimental conditions. LOQ is the lowest concentration of analyte in a sample that can be determined with acceptable precision.

In order to determine LOD and LOQ,

The LOD was estimated from the set of 3 calibration curves used to determination linearity.

The LOD may be calculated as,

$$\text{LOD} = 3.3 \times (\text{SD}/\text{Slope})$$

Where, SD= Standard deviation of Y-intercepts of calibration curve.

Slope = Mean slope of the calibration curve.

The LOQ was estimated from the set of 3 calibration curves used to determine linearity.

The LOQ may be calculated as,

$$\text{LOQ} = 10 \times (\text{SD}/\text{Slope})$$

Where, SD= Standard deviation of Y-intercepts of calibration curve.

6. Robustness:

Robustness of the method was studied by making small deliberate changes in few parameters.

Teneligliptin, Metformin and Pioglitazone (1.6, 40 and 1.2µg/ml respectively) drug solution was taken and injected by applying little deliberate changes of the following method conditions and evaluated by RSD.

- i. Column Temperature: ± 1 °C
- ii. Flow rate: ± 0.1 ml/min
- iii. Mobile Phase pH : ± 0.1

Acceptance criteria:

Number of theoretical plates for the analyte peak should not be less than 2000.

Asymmetry value for the analyte peak should not be more than 2.0.

RSD for the analyte peak should not be more than 2.0%.

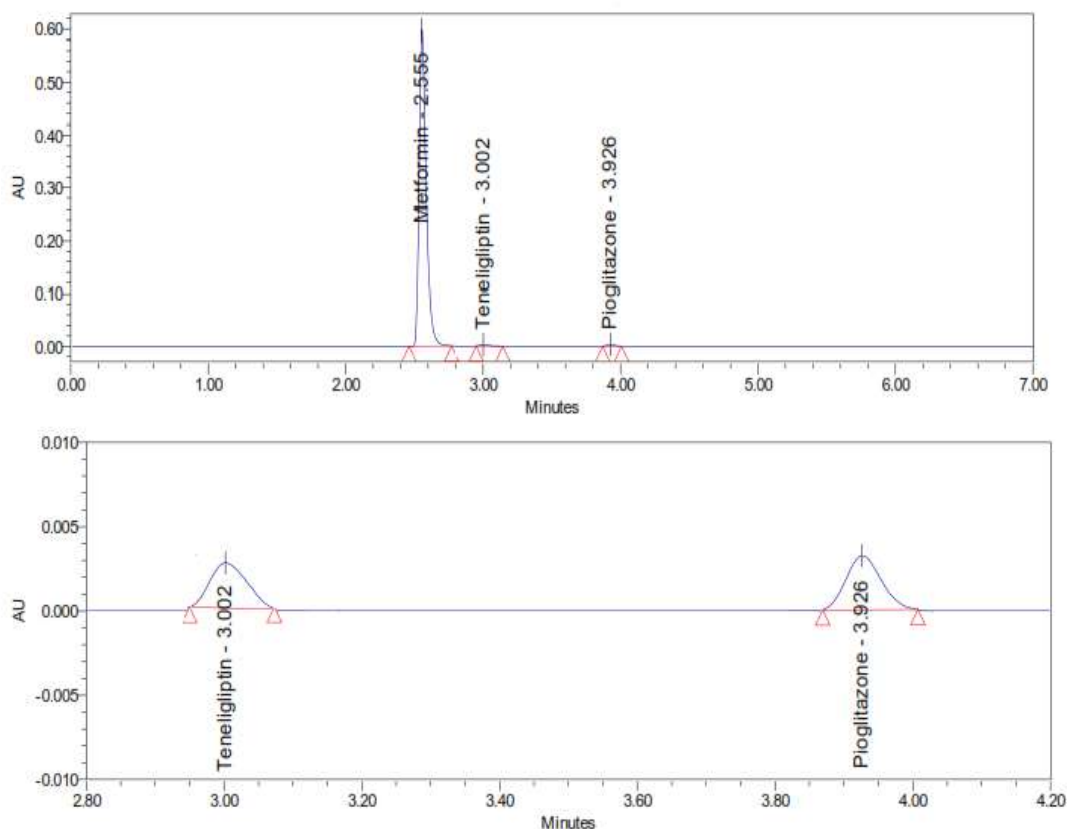
7. Application of method on marketed products:

(Label claim: Teneligliptin - 20mg, Metformin - 500mg and Pioglitazone - 15mg)

Twenty tablets were weighed; average weight was calculated and tablets were powdered finely. Tablet Powder equivalent to 2mg of Teneligliptin, 50mg of Metformin and 1.5mg of Pioglitazone were added into 50ml of volumetric flask. Teneligliptin (400µg/ml), Metformin (1000µg/ml) and Pioglitazone (300µg/ml). Volume was made up to the mark with diluent. 1ml of above solution was transferred to 10ml volumetric flask. Volume was made up to the mark with diluent, which gives Teneligliptin (4µg/ml), Metformin (100µg/ml) and Pioglitazone (3µg/ml). 4ml of above solution was transferred to 10ml volumetric flask. Volume was made up to the mark with diluent, which gives Teneligliptin (1.6µg/ml), Metformin (40µg/ml) and Pioglitazone (1.2µg/ml). The quantification was carried out by keeping these values to be straight line equation of calibration curve.

II. RESULTS AND DISCUSSION

The developed UPLC method involves separation of Teneligliptin, Metformin and Pioglitazone on a Welch C18 column (4.6 × 150 mm, 2 µm) at an ambient column temperature. The optimized mobile phase consists of Methanol: Acetonitrile: phosphate buffer (pH adjusted to 2.5) (70:05:25 % v/v/v) with a flow rate of 0.5 ml/min and UV detection at 238 nm. Retention time was 0.30 min for Teneligliptin, 0.25 min for Metformin and 0.39 min for Pioglitazone. The optimized method was validated as per ICH guidelines. Chromatogram of Teneligliptin, Metformin and Pioglitazone is shown in Fig. 7.



Peak Results

Name	RT	Area	Height	Symmetry Factor	Resolution	USP Tailing	USP Plate Count	Purity1 Threshold	Purity1 Angle
1 Metformin	2.555	2127797	598770	1.45		1.45	12110	0.246	0.171
2 Teneiglipitin	3.002	10957	2681	1.66	4.53	1.66	11932	1.149	1.009
3 Pioglitazone	3.926	12431	3210	1.16	9.11	1.16	27414	0.678	0.670

Fig.7 UPLC Chromatogram of Metformin, Teneiglipitin and Pioglitazone [Methanol: ACN: Potassium Phosphate buffer (pH2.5) (70:05:25 %v/v/v)]

Linearity

The standard curve for Teneiglipitin, Metformin and Pioglitazone were linear over the investigated concentration range 0.08-4µg/ml for Teneiglipitin, 2-100µg/ml for Metformin and 0.06-3µg/ml for Pioglitazone with a percent relative standard deviation (% RSD) of not more than 2 based on seven successive readings. Correlation coefficient value should not be less than 0.995 for given range. Correlation coefficient value were found to be 0.9996, 0.9999 and 0.9998 for Teneiglipitin, Metformin and Pioglitazone respectively, which is greater than 0.995. Hence, the method is linear within the range.

Precision

The precision of an analytical method is the degree of agreement among individual test results obtained when the method is applied to multiple sampling of a homogenous sample. Precision studies of proposed method were determined by repeatability, intra-day and inter-day precision. For the repeatability, RSD of the assay of six sample preparations should not be more than 2%. The obtained RSD was found to be 0.40%, 0.19% and 0.36% for Teneiglipitin, Metformin and Pioglitazone respectively. which are well within the limit of acceptance criteria. While for the intermediate precision of the method, the same procedure was followed on a same day at specific interval and on different day. RSD for intraday

precision were found to be in the range of 0.33-0.52%, 0.20-0.55% and 0.43-0.77% for Teneligliptin, Metformin and Pioglitazone respectively. RSD for interday precision were found to be in the range of 0.28-0.47%, 0.27-0.69% and 0.49-0.66% for Teneligliptin, Metformin and Pioglitazone respectively. which also well within the limit of acceptance criteria and absolute difference between mean assay value of method precision and intermediate precision was found to be less than 2.0 % which is within the limit of acceptance criteria. Hence, the method can be termed as precise. (shown in table-1 to 9).

Accuracy

The result of this study was found to be within the acceptance criteria of method validation (i.e. the recovery is 98% - 102% and the RSD is NMT 2.0%). Teneligliptin, Metformin and Pioglitazone which were found to be as 98.52-101.52%, 99.17-99.43% and 98.63-101.31 respectively. This proves that the test method is accurate for the estimation of Teneligliptin, Metformin and Pioglitazone. (shown in table-10 to 12).

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD for Teneligliptin, Metformin and Pioglitazone was found to be 0.04µg/ml, 0.32µg/ml and 0.19µg/ml respectively. Similarly LOQ for Teneligliptin, Metformin and Pioglitazone was found to be 0.12µg/ml, 0.97µg/ml and 0.58µg/ml respectively. (shown in table-13,14).

Robustness

The robustness study is used to demonstrate the method's efficiency in the face of purposeful changes in conventional method factors, such as Column temp. , flow rate, pH. The assay obtained following the changes suggested was compared to the assay obtained under normal conditions. The test difference should not be greater than 2%, according to the approval requirements. The gained outcomes are well within the acceptable ranges. As a result, the approach may be described as robust (shown in table-15 to 17).

Assay

By taking the mean of three determinations, By UPLC method %assay was found 100.70% for Teneligliptin, 100.50% for Metformin and 99.98% for Pioglitazone. So the developed method can be used for routine analysis. (shown in table-18).

Table 1 Repeatability data of Teneligliptin

Teneligliptin (1.6µg/ml)				
Sr. No.	Conc. (µg/ml)	Area	Mean ± S.D (n=6)	RSD (%)
1	1.6	10915	10934.5	0.40
		10881	±	
		10957	44.81852	
		10895		
		10961		
		10988		

Table 2 Repeatability data of Metformin

Metformin (40 µg/ml)				
Sr. No.	Conc. (µg/ml)	Area	Mean ± S.D (n=6)	RSD (%)
1	40	2099726	2099831	0.19
		2101650	±	
		2107065	4069.161	
		2097004		

		2097218	
		2096321	

Table 3 Repeatability data of Pioglitazone

Pioglitazone (1.2µg/ml)				
Sr. No.	Conc. (µg/ml)	Area	Mean ± S.D (n=6)	RSD (%)
1	1.2	12474	12463.3 ± 44.2482	0.36
		12413		
		12485		
		12404		
		12509		
		12496		

Intraday Precision:

Table 4 Intraday Data for Teneligliptin

Teneligliptin			
SR. NO.	Conc. (µg/ml)	Area Mean ± SD (n=3)	RSD (%)
1	0.8	5688 ±29.81	0.52
2	1.6	10946.33 ±48.211	0.44
3	2.4	16049 ±54.14	0.33

Table 5 Intraday Data for Metformin

Metformin			
SR. NO.	Conc. (µg/ml)	Area Mean ± SD (n=3)	RSD (%)
1	20	1047164 ±5820.008	0.55
2	40	2088456 ±7363.807	0.35
3	60	3145300 ±6335.095	0.20

Table 6 Intraday Data for Pioglitazone

Pioglitazone			
SR. NO.	Conc. (µg/ml)	Area Mean ± SD (n=3)	RSD (%)
1	0.6	6213 ±47.88	0.77
2	1.2	12405.33 ± 65.85	0.53
3	1.8	18506 ±80.31	0.43

Interday Precision:

Table 7 Interday Data for Teneligliptin

Teneligliptin			
SR. NO.	Conc. (µg/ml)	Area Mean ± SD (n=3)	RSD (%)
1	0.8	5678.66 ±27.02	0.47
2	1.6	10917.33 ±46.11	0.42
3	2.4	16021 ±46.35	0.28

Table 8 Interday Data for Metformin

Metformin			
SR. NO.	Conc. (µg/ml)	Area Mean ± SD (n=3)	RSD (%)
1	20	1052572 ±7327.06	0.69
2	40	2095128 ±8072.47	0.38
3	60	3143148 ±8708.59	0.27

Table 9 Interday Data for Pioglitazone

Pioglitazone			
SR. NO.	Conc. (µg/ml)	Area Mean ± SD (n=3)	RSD (%)
1	0.6	6211.66 ±41.47	0.66
2	1.2	12407 ±63.50	0.51
3	1.8	18424 ±91.06	0.49

Accuracy:

Table 10 Recovery data for Teneligliptin

SR. NO.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery
1	50	1.6	0.8	2.436	101.52
2		1.6	0.8	2.423	100.98
3		1.6	0.8	2.419	100.81
1	100	1.6	1.6	3.200	100.01
2		1.6	1.6	3.171	99.09
3		1.6	1.6	3.195	99.85
1	150	1.6	2.4	3.943	98.59
2		1.6	2.4	3.953	98.83
3		1.6	2.4	3.941	98.52

Table 11 Recovery data for Metformin

SR. NO.	Conc. Level (%)	Sample Amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery
1	50	40	20	59.630	99.383
2		40	20	59.617	99.362
3		40	20	59.616	99.360
1	100	40	40	79.448	99.310
2		40	40	79.337	99.172
3		40	40	79.392	99.241
1	150	40	60	99.436	99.436
2		40	60	99.325	99.325
3		40	60	99.353	99.353

Table 12 Recovery data for Pioglitazone

SR. NO.	Conc. Level (%)	Sample Amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery
1	50	1.2	0.6	1.7810	98.94
2		1.2	0.6	1.7753	98.63

3		1.2	0.6	1.7897	99.42
1	100	1.2	1.2	2.3945	99.77
2		1.2	1.2	2.3833	99.30
3		1.2	1.2	2.3927	99.69
1	150	1.2	1.8	3.0143	100.47
2		1.2	1.8	3.0393	101.31
3		1.2	1.8	3.0123	100.41

LOD AND LOQ:

Table 13 Limit of detection data for Teneligliptin, Metformin and Pioglitazone

Teneligliptin	Metformin	Pioglitazone
LOD = 3.3 x (SD / Slope) = 3.3 x (80/6479) = 0.04µg/ml	LOD = 3.3 x (SD / Slope) = 3.3 x (5167.74/52752) = 0.32µg/ml	LOD = 3.3 x (SD / Slope) = 3.3 x (598.15/10242) = 0.19µg/ml

Table 14 Limit of Quantitation data for Teneligliptin, Metformin and Pioglitazone

Teneligliptin	Metformin	Pioglitazone
LOQ = 10 x (SD / Slope) = 3.3 x (80/6479) = 0.12µg/ml	LOQ = 10 x (SD / Slope) = 10 x (5167.74/52752) = 0.97µg/ml	LOQ = 10 x (SD / Slope) = 3.3 x (598.15/10242) = 0.58µg/ml

Robustness:

Table 15 Robustness data for Teneligliptin

SR. NO.	Area at Column Temp. -1 °C	Area at Column Temp. +1 °C	Area at Flow rate (-0.1 ml/min)	Area at Flow rate (+0.1ml/min)	Area at pH (-0.1)	Area at pH (+0.1)
1	11705	11130	11689	11067	11786	11192
2	11740	11193	11726	11315	11791	11158
3	11644	11156	11773	11329	11713	11121
AVG. Area	11599.33	11331	11615.83	11369.67	11632.83	11329.67
SD	130.61	201.12	144.88	186.10	161.31	202.74
%RSD	1.126	1.774	1.247	1.636	1.386	1.789

Condition	Mean Area	Mean	SD	RSD
Column Temp.	24	11599.33	11477.55	135.87
	25	11502.33		
	26	11331.23		
Flow rate (ml/min)	0.45	11615.83	11495.94	123.20
	0.5	11502.33		
	0.55	11369.67		
pH of Mobile phase	2.4	11632.83	11488.28	152.07
	2.5	11502.33		
	2.6	11329.67		
				1.183
				1.071
				1.323

Table 16 Robustness data for Metformin

SR. NO.	Area at Column Temp. -1 °C	Area at Column Temp. +1 °C	Area at Flow rate (-0.1 ml/min)	Area at Flow rate (+0.1ml/min)	Area at pH (-0.1)	Area at pH (+0.1)
1	2152175	2084792	2175308	2087560	2173551	2086454
2	2159332	2089754	2167904	2091156	2160536	2088946
3	2161587	2097546	2157011	2099850	2165866	2095654
AVG. Area	2128729.83	2095230	2133251	2096309	2133206	2095057
SD	32686.95	9642.30	37835.76	9059.35	37565.55	9349.58
%RSD	1.535	0.460	1.773	0.432	1.760	0.446

Condition		Mean Area	Mean	SD	%RSD
Column Temp.	24	2128729.83	2107907	18174.92	0.862
	25	2099762			
	26	2095230			
Flow rate (ml/min)	0.45	2133251	2109774	20405.29	0.967
	0.5	2099762			
	0.55	2096309			
pH of Mobile phase	2.4	2133206	2109342	20801.02	0.986
	2.5	2099762			
	2.6	2095057			

Table 17 Robustness data for Pioglitazone

SR. NO.	Area at Column Temp. -1 °C	Area at Column Temp. +1 °C	Area at Flow rate (-0.1 ml/min)	Area at Flow rate (+0.1ml/min)	Area at pH (-0.1)	Area at pH (+0.1)
1	12574	11955	12702	11888	12552	12190
2	12653	12089	12632	12113	12601	11946
3	12499	12248	12527	12296	12540	12065
AVG. Area	12479.33	12240.33	12501.83	12241.17	12473.83	12225.17
SD	134.84	194.67	157.18	213.80	122.47	201.81
%RSD	1.080	1.590	1.257	1.746	0.981	1.650

Condition		Mean Area	Mean	SD	%RSD
Column Temp.	24	12479.33	12367.66	120.26	0.972
	25	12383.33			
	26	12240.33			
Flow rate (ml/min)	0.45	12501.83	12375.44	130.51	1.054
	0.5	12383.33			
	0.55	12241.17			
pH of Mobile phase	2.4	12473.83	12360.78	125.85	1.018
	2.5	12383.33			
	2.6	12225.17			

Table 18 Assay of Teneligliptin, Metformin and Pioglitazone

Sr. No.	Teneligliptin		Metformin		Pioglitazone	
	Area of samples	Area of samples	Area of samples	%Assay	Area of samples	%Assay
1	10927	100.4195	2112758	100.1149	12458	100.2092

2	10893	100.0915	2111856	99.4858	12415	99.8594
3	11007	101.1911	2111998	99.6806	12491	100.4777
	Avg. Assay	100.5674	Avg. Assay	99.7604	Avg. Assay	100.1821
	SD	0.5645	SD	0.3220	SD	0.3100
	RSD of Assay	0.5613	RSD of Assay	0.3228	RSD of Assay	0.3094

III. CONCLUSION:

From above observations, it can be concluded that developed validation of Teneligliptin, Metformin and Pioglitazone in tablets by UPLC is, specific, linear, accurate, precise and robust. Thus above developed UPLC method can be applied for routine analysis.

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