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Development and Validation of HPTLC Method for Simultaneous Estimation of Teneligliptin and Pioglitazone from Their Combined Dosage Form

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ABSTRACT

A simple, accurate and precise HPTLC method for simultaneous determination of Teneligliptin hydrobromide hydrate (TEN) and Pioglitazone hydrochloride (PIO) in tablet dosage form has been developed using Camag TLC system (Switzerland) comprising of CAMAG Linomat 5 applicator. The chromatographic separation was carried out using Aluminum TLC plate, silica gel coated with fluorescent indicator F_{254}, (20×10 cm) as stationary phase and Chloroform: Methanol: Toluene (6:1.5:2.5 v/v/v) as mobile phase. Using the developed method, Rf value was found to be 0.466±0.020 for TEN and 0.710±0.030 for PIO. The calibration curve was found to be linear between 400-2000 ng/band for TEN and 300-1500 ng/band for PIO with correlation coefficients value (r^2) of 0.9973 and 0.9978 for TEN and PIO respectively. The above method was validated as per ICH guidelines. The proposed method is able to accurately determine the drug content of marketed formulations.

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INTRODUCTION

The development and validation of analytical methods is a crucial component of pharmaceutical quality control. Diabetes is a chronic disease that happens when either the pancreas fails to produce enough insulin or the body is unable to utilize the insulin it does produce. The hormone insulin is responsible for regulating blood glucose. Uncontrolled diabetes often leads to hyperglycemia, which is another term for elevated blood glucose or blood sugar and can cause significant damage to many of the body's systems, mostly the nerves and blood vessels, over time.[1]

Teneligliptin and Pioglitazone belong to oral antidiabetic drugs. Teneligliptin, a newly discovered oral dipeptidyl peptidase-4 inhibitor, is effective in managing type 2 diabetes mellitus in adults along with diet and exercise.[2] Pioglitazone is a Thiazolidinedione (TZD) drug that addresses the primary problem of insulin resistance in type 2 diabetes mellitus.[3]

High-performance thin-layer chromatography is an advanced instrumentation technique that combines all the capabilities of thin-layer chromatography. Automation, scanning, full optimization, selective detection principles, minimal sample preparation, and hyphenation make it a powerful analytical tool for obtaining chromatographic information from complex mixtures in pharmaceuticals, natural products, clinical samples, food products, and more.[4]

The literature review revealed that very few analytical methods[11-15] were reported to simultaneously estimate Teneligliptin and Pioglitazone in the tablet dosage form.

The study aimed to develop a simple, precise, accurate HPTLC method for simultaneously estimating Teneligliptin and Pioglitazone in pharmaceutical dosage forms. The method was validated according to ICH guidelines.[6]

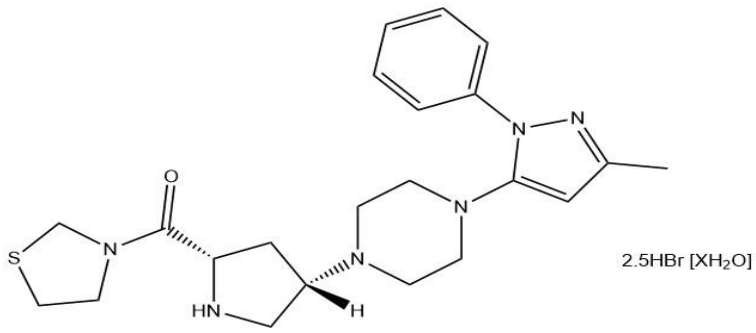

Figure 1: Teneligliptin hydrobromide hydrate

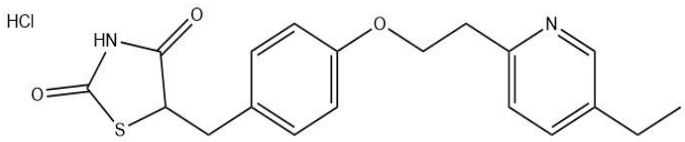

Figure 2: Pioglitazone Hydrochloride

2.0 MATERIALS AND METHODS

2.1 Materials

Standard materials of Teneligliptin and Pioglitazone were obtained as gift samples from Synokem Pharmaceuticals (India) Pvt, Ltd., Chennai. Zita Pio tablets containing 20 mg of Teneligliptin and 15 mg of Pioglitazone were selected for sample analysis, procured from the local market. Silica gel 60 F_{254} TLC plates (20x10 cm, layer thickness 250 µm thickness, Merck, Germany) was used as stationary phase during analysis. Chloroform, Methanol, Toluene, were used as solvents to prepare the mobile phase. All the chemicals were of HPLC grade (Merck Life Sciences) used without further purification.

Instrumentation and chromatographic conditions

The samples were spotted in the form of bands of width 7 mm with 100 µl sample syringe (Hamilton) on precoated silica gel aluminium plate 60 F_{254} (20 x 10 cm) using a Camag Linomat 5 (Switzerland) sample applicator. A constant application rate of 150 nl/sec was employed. The slit dimension was kept at 6×0.45 mm. The mobile phase consists of Chloroform: Methanol: Toluene (6:1.5:2.5 v/v/v). Linear ascending development was carried out in 20×10 cm twin trough glass chamber. The optimized chamber saturation time for mobile phase was 20 min, at temperature (25±2°) and relative humidity (60±5%). Densitometric scanning was performed on a Camag TLC Scanner 4 equipped with VisionCATS software version 3.0 at 254 nm. The source of radiation utilized was deuterium lamp. Evaluation was performed using peak area with linear regression. Optimization of chromatographic condition were based upon various preliminary trails carried as tabulated in table 1 and 2.

Preparation of standard and sample solutions:

Standard stock solutions

TEN and PIO standard stock solutions were prepared separately by accurately weighing 4 mg and 3 mg of TEN and PIO, respectively, into two separate 10 ml standard flasks. Methanol was added in half the volume and the solutions were sonicated for 10 min before the final volume was made up with methanol. Teneligliptin and Pioglitazone concentration of 0.4 mg/ml (400 µg/ml) and 0.3 mg/ml (300 µg/ml) were obtained, respectively.

Preparation of sample solution

Weighed and powdered twenty Zita Pio tablets containing Teneligliptin (20 mg) and Pioglitazone (15 mg). An amount of tablets powder equivalent to 20 mg Teneligliptin was weighed and placed in a 25 ml volumetric flask, 10 ml methanol was added and sonicated for 10 min, and the volume was made to 25 ml with methanol and thoroughly mixed. The solution above has been filtered. The filtrate was diluted appropriately before being used for further investigation.

1 µl of the above solution was spotted on a precoated TLC plate and developed. After development, the plate was dried and scanned at 254 nm. The sample's peak area was measured.

Method validation:

Linearity

Appropriate aliquots of Teneligliptin and Pioglitazone standard stock solutions were diluted up to the mark with Methanol in five different 10 ml volumetric flasks to obtain final concentrations ranging from 400-2000 µg/ml for Teneligliptin and 300-1500 µg/ml for Pioglitazone.

On the precoated TLC plate, 1 µl of the above solutions were spotted. The development technique is ascending development process. After drying, the plate was scanned at 254 nm. The peak regions were measured and calibration curves were built in the concentration ranges of 400-2000 ng/band for Teneligliptin and 300-1500 ng/band for Pioglitazone.

Accuracy

Recovery studies using the conventional addition method was performed to test the accuracy at 80 %, 100 %, and 120 % of target concentration levels. The amount of drug recovered was calculated using the following formula.

$$\%\text{Recovery} = \frac{Amount\ recovered - Amount\ added}{Amount\ present} * 100$$

Precision

The intra-day and inter-day precision investigations (intermediate precision) were conducted by calculating the equivalent responses six times on the same day and three times on three distinct days at 100% concentration (800 µg/ml Teneligliptin and 600 µg/ml Pioglitazone). 1 µl of the aforesaid solution were spotted six times on the precoated TLC plate and developed. After development, the plates were dried and scanned at 254 nm and the peak areas were recorded.

The limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations below:

$$LOD = 3.3\frac{\sigma}{S} \qquad LOQ = 10\frac{\sigma}{S}$$

where, σ: standard of y-intercept and S: slope of calibration curve.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The solubility studies revealed Teneligliptin and Pioglitazone were soluble in methanol, and hence the method development started with 100% methanol as the mobile phase. Further solvents of varying polarity with different combinations were tested to assess the chromatographic behaviour of the selected analytes. With the previous knowledge and the trial runs conducted, diverse mobile phase systems in different ratios were explored, and the results are depicted in table 2. A good, reasonable separation with compact spots and ideal Rf values of 0.466 and 0.710 for Teneligliptin and Pioglitazone, respectively, was obtained with Chloroform: Methanol: Toluene as the mobile phase in a ratio of 6:1.5:2.5.

Table 1: Initial trials with different solvents

Sl No	Solvent system	Ratio	Teneligliptin	Pioglitazone
1	Methanol	-	Movement of spot to the solvent front	Movement of spot to the solvent front
2	Hexane:Ethyl Acetate	2:8	Spot movement was not observed	Movement of spot to the solvent front
3	Acetone:Diethyl Ether	7:3	Spot movement was not observed	Movement of spot to the solvent front
4	Methanol:Ethyl Acetate	9:1	Partial movement of the spot just above the baseline	Movement of spot to the solvent front
5	Methanol: Toluene	3:7	Movement of spot to the solvent front	Movement of spot to the solvent front
6	Methanol:Ethyl Acetate :Toluene	6:3:1	Movement of the spot below the solvent front	Movement of spot to the solvent front
7	Butanol:Chloroform :Toluene	3:5:2	Spot movement was not observed	Movement of the spot below the solvent front
8	Chloroform: Methanol :Ethyl Acetate	7:2:1	Movement of the spot below the solvent front	Movement of spot to the solvent front
9	Chloroform: Methanol :Toluene	6:2:2	Movement of the spot below the solvent front	Movement of the spot below the solvent front

Table 2: Optimization of Mobile phase

Sl No	Solvent system	Ratio
1	Chloroform :Methanol :Toluene	7:1:2
2	Chloroform :Methanol :Toluene	6:2.5:1.5
3	Chloroform :Methanol :Toluene	6:1.5:2.5

Chloroform: Methanol: Toluene (**6:1.5:2.5**) was chosen as the mobile phase, which gave a chromatogram with good resolution for Teneligliptin and Pioglitazone.

Determination Rf value

Table 3: Rf values of the drugs

Drug	Rf
Teneligliptin	0.466
Pioglitazone	0.710

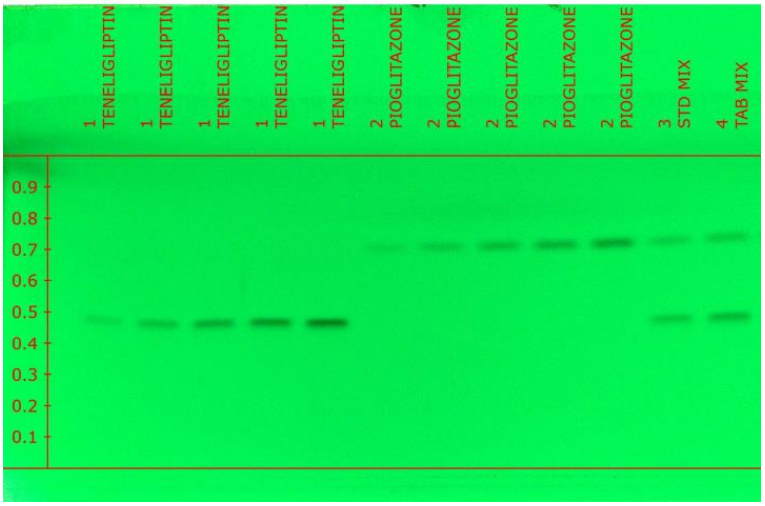

Figure 3: Photograph of Developed HPTLC Plate in Short-Wave UV Light (254nm)

The figure 3 shows the photograph of developed plate in UV light of 254nm. The first 5 bands represent the band of Teneligliptin in a concentration range from 400 – 2000 ng/band. The next 5 bands represent Pioglitazone in a concentration range from 300 – 1500 ng/band. The last 2 bands represent the standard drug mixture and tablet mixture in a concentration of 800 ng/band for Teneligliptin and 600 ng/band for Pioglitazone.

Chromatogram of standard drug mixture

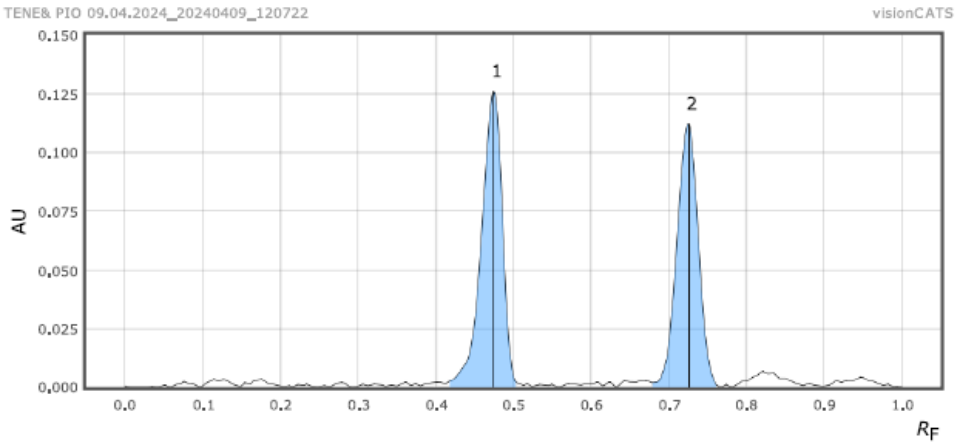

Figure 4: HPTLC Chromatogram of Teneligliptin Hydrobromide Hydrate and Pioglitazone in Standard Mixture

Assay of Teneligliptin and Pioglitazone in marketed formulation (Tablets)

The novel approach was employed to assess the Zita Pio tablets, a commercial product containing Teneligliptin (20 mg) and Pioglitazone (15 mg). The sample was treated in accordance with the procedure for analysing Teneligliptin and Pioglitazone. The densitogram

of the tablet sample revealed two distinct peaks, with calculated Rf values of 0.466 and 0.710 for Teneligliptin and Pioglitazone, respectively, indicating non-interaction among the tablet excipients. The quantities of Teneligliptin and Pioglitazone present were ascertained by comparing the peak areas of the sample to the standard, yielding a % RSD of less than 2%. Densitogram for the assay is depicted in Figure 5.

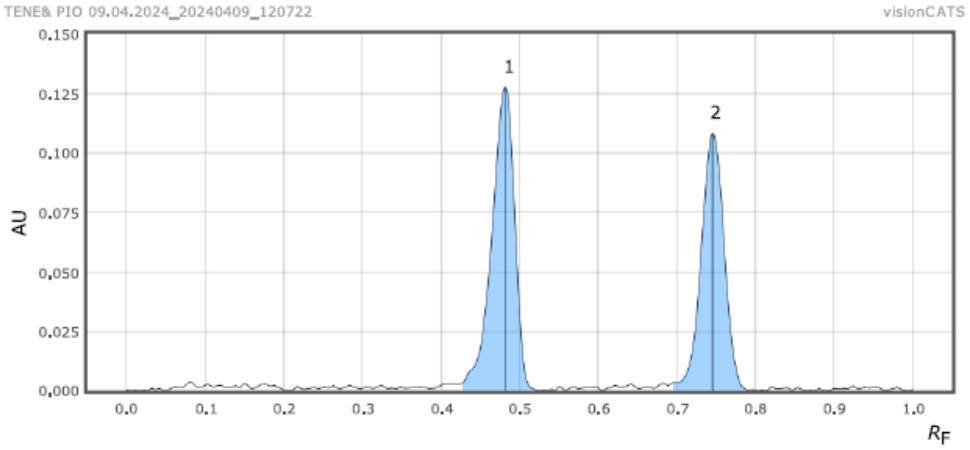

Figure 5: HPTLC Chromatogram of Teneligliptin Hydrobromide Hydrate and Pioglitazone in Sample

Table 4: Assay of Teneligliptin and Pioglitazone in marketed formulation

Drug	Label claim (mg)	Amount estimated (mg)	% Assay
Teneligliptin	20	20.38039	101.90%
Pioglitazone	15	14.74813	98.32%

VALIDATION

Linearity and range

Peak areas were discovered to have a stronger linear connection with concentration than peak heights. The r^2 for Teneligliptin was 0.9973, whereas the r^2 for Pioglitazone was 0.9978. Calibration graphs were created for Teneligliptin in the concentration range of 400-2000 ng/band and Pioglitazone in the concentration range of 300-1500 ng/band. The correlation coefficients, y-intercepts, and slopes of the two drugs, regression lines were calculated.

The linearity data are tabulated in table 5 and linearity plots were represented in fig. 6 and 7, respectively. LOD and LOQ were calculated from the linearity data and the results were tabulated in table 6.

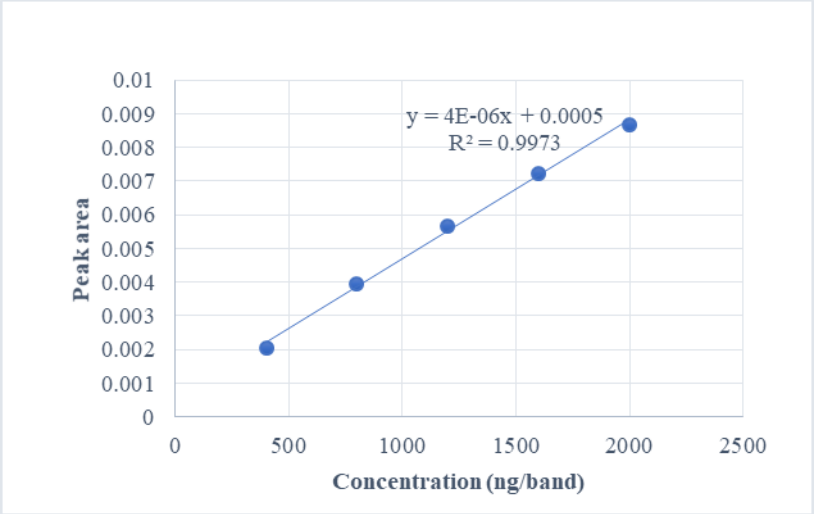

Fig.6: Calibration Plot of Teneligliptin (Concentration v/s Peak area)

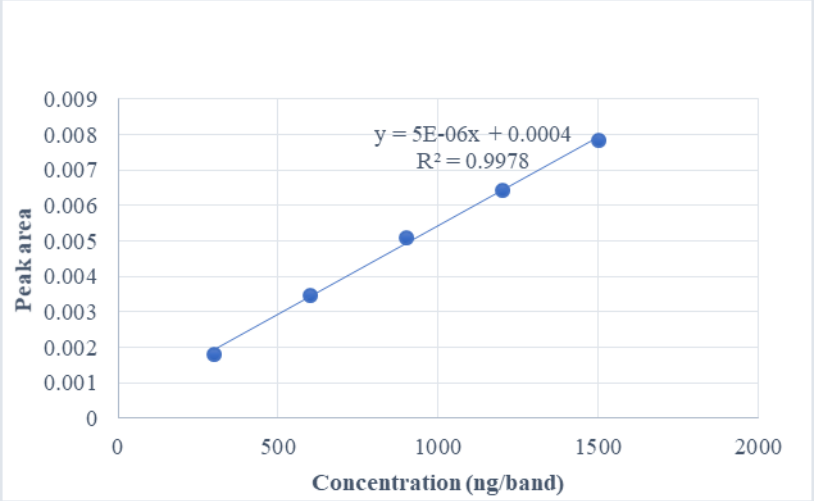

Fig.7: Calibration Plot of Pioglitazone (Concentration v/s Peak area)

Table 5: Linearity of Teneligliptin and Pioglitazone

S.no	Drug	Concentration (ng/band)	Peak area
1	Teneligliptin	400	0.00205
		800	0.00395
		1200	0.00566
		1600	0.00722
		2000	0.00869
2	Pioglitazone	300	0.00180
		600	0.00346
		900	0.00508
		1200	0.00641
		1500	0.00783

Table 6: LOD and LOQ data

Drug	Linearity range	Regression equation	R^2	Slope	Intercept	LOD (ng/band)	LOQ (ng/band)
Teneligliptin	400-2000	y= 4E-06x + 0.0005	0.9973	4.14E-06	0.0005	108.3296	328.2714
Pioglitazone	300-1500	y= 5E-06x + 0.0004	0.9978	5.00E-06	0.0004	73.5833	222.9798

Accuracy

The method's accuracy was evaluated at 80%, 100%, and 120% of the target concentration. The accuracy was tested, the results were tabulated and displayed in table 7.

Table 7a: Data of Recovery study of Teneligliptin and Pioglitazone

Sl.no.	% Recovery	Teneligliptin	Pioglitazone
		Peak Area	Peak Area
1	80%	0.00326	0.00292
2		0.00325	0.00291
3		0.00321	0.00286
1	100%	0.00391	0.00363
2		0.00394	0.00373
3		0.0039	0.00369
1	120%	0.00451	0.00427
2		0.00449	0.00422
3		0.00456	0.00424

Table 7b: Results of Accuracy study of Teneligliptin and Pioglitazone

Drug	Levels of recovery (%)	Amount initially present (µg/ml)	Amount added (µg/ml)	Recovery (%)	%RSD
Teneligliptin	80	800	640	103.05%	0.8166
	100	800	800	99.66%	0.5315
	120	800	960	95.84%	0.7977
Pioglitazone	80	600	480	101.14%	1.1097
	100	600	600	102.89%	1.3665
	120	600	720	98.77%	0.5930

Precision

The method's precision was evaluated using the relative standard deviation (RSD) of the peak area. The findings indicated that the intra-day and inter-day variability at 800 µg/ml concentrations for Teneligliptin and 600 µg/ml for Pioglitazone met the acceptable criteria.

The coefficients of variation for the intra-day and inter-day precision of the method were established to be below 2% for both pharmaceuticals. A comprehensive synthesis of the precision study results is presented in Tables 8 and 9.

Table 8a: Data of Repeatability study of Teneligliptin and Pioglitazone

Sl. No.	TEN	PIO
	Peak Area	Peak Area
1	0.00402	0.00369
2	0.00394	0.00357
3	0.00391	0.00351
4	0.00399	0.00354
5	0.00395	0.00351
6	0.00397	0.00355

Table 8b: Results of Repeatability study

Drug	Amount obtained (µg/ml)	% Drug content
TEN	20.5852	102.93%
	20.1756	100.88%
	20.0219	100.11%
	20.4316	102.16%
	20.2268	101.13%
	20.3292	101.65%
PIO	14.9576	99.72%
	14.7062	98.04%
	14.7481	98.32%
	14.8319	98.88%
	14.7062	98.04%
	14.8738	99.16%

Table 8c: Repeatability Study- Statistical Validation

Drug	Method	Mean (n=6)	Standard deviation	RSD (%)
Teneligliptin Hydrobromide Hydrate	Area wise	101.48%	0.00994	0.97937
Pioglitazone	Area wise	98.69%	0.00677	0.68551

Table 9a: Inter-day Precision Data- Teneligliptin

Sl No	Day	Peak Area	Drug recovered	Percentage label claim per tablet(%)
1	Day 1	0.00395	20.2268	101.13%
2		0.00394	20.1756	100.88%
3		0.00395	20.2268	101.13%
1	Day 2	0.00398	20.3804	101.90%
2		0.00402	20.5852	102.93%
3		0.00396	20.2780	101.39%
1	Day 3	0.00392	20.0731	100.37%
2		0.00399	20.4316	102.16%
3		0.00408	20.8925	104.46%

Table 9b: Inter-day precision Data-Pioglitazone

Sl No	Day	Peak Area	Drug recovered	Percentage label claim per tablet(%)
1	Day 1	0.00364	15.2509	101.67%
2		0.00358	14.9995	100.00%
3		0.00359	15.0414	100.28%
1	Day 2	0.00361	15.1252	100.83%
2		0.00357	14.9576	99.72%
3		0.00356	14.9157	99.44%
1	Day 3	0.00363	15.2090	101.39%
2		0.00365	15.2928	101.95%
3		0.00358	14.9995	100.00%

Table 9c: Inter-day precision- Statistical validation

Drug	Concentration (ng/band)	Method	Mean (n=9)	SD	% RSD
Teneligliptin	800	Area	101.82%	0.0125	1.2255
Pioglitazone	600		100.59%	0.0091	0.9046

CONCLUSION

Only a few simple and rapid methods have been reported for the simultaneous estimation of Teneligliptin and Pioglitazone. To address this, an HPTLC (High-Performance Thin-Layer Chromatography) method was developed and validated for determining Teneligliptin and Pioglitazone in tablet formulations using pre-coated silica gel HPTLC plates and a simple

mobile phase. The optimized method was found to be simple, quick, selective, sensitive, and suitable for simultaneous determination of both compounds. HPTLC offers several advantages over liquid chromatographic methods, such as the ability to analyze a sample and a standard on the same plate, short system equilibrium time, multiple/repeated scanning of chromatograms, short run time, low solution consumption, and no prior solvent treatment such as filtration and degassing. Therefore, this method can be routinely applied for the simultaneous estimation of Teneligliptin and Pioglitazone.

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CONFLICT OF INTEREST

There is no conflict of interest.

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