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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF TENELIGLIPTIN, METFORMIN AND PIOGLITAZONE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Objective: This study aimed to develop a straightforward, precise, and accurate method for the analysis of Teneligliptin, Metformin, and Pioglitazone in pharmaceutical dosage forms. **Method**: The separation was achieved using a Waters UPLC system with a Welch C18 column (150×4.6mm, 2 µm) and a mobile phase consisting of Methanol: ACN: Phosphate Buffer pH 2.5 (70:05:25), adjusted with orthophosphoric acid. The flow rate was set at 0.5 mL/min, and detection was performed at 238 nm using a photodiode array detector. **Result**: The complete validation of the analytical method was conducted following ICH guidelines. Recovery studies were performed at concentrations ranging from 50% to 150%, yielding results between 98% and 102%. The linearity ranges were determined as 0.08-4 µg/mL for Teneligliptin, 2-100 µg/mL for Metformin, and 0.06-3 µg/mL for Pioglitazone, with linear regression curves showing R2 values of 0.999. The method exhibited limits of detection (LOD) and quantification (LOQ) of 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. Retention times were 3.00 min for Teneligliptin, 2.55 min for Metformin, and 3.92 min for Pioglitazone. Intra-day and inter-day relative standard deviations were below 2%, confirming the method's precision. Ruggedness and robustness evaluations, conducted according to ICH guidelines, also demonstrated satisfactory results. **Conclusion:** The developed UPLC method is suitable for the simultaneous estimation of Teneligliptin, Metformin, and Pioglitazone in pharmaceutical dosage forms, offering a reliable approach for routine analysis.

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

INTRODUCTION

Teneligliptin, with the IUPAC name {(2S,4S)-4-[4-(5-Methyl-2-phenylpyrazol-3-yl)piperazin-1-yl]pyrrolidin-2-yl}-(1,3-thiazolidin-3-yl)methanone, has the chemical formula C22H30N6OS (Fig. 1). It belongs to the class of Dipeptidase-4 (DPP-4) inhibitors, which are

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used as anti-diabetic drugs. The mechanism of action of DPP-4 inhibitors involves increasing incretin levels, which inhibit glucagon release. This action results in increased insulin secretion, decreased gastric emptying, and ultimately leads to reduced blood glucose levels. DPP-4 inhibitors like Teneligliptin work by modulating these processes to help manage blood glucose levels in diabetic patients[1].

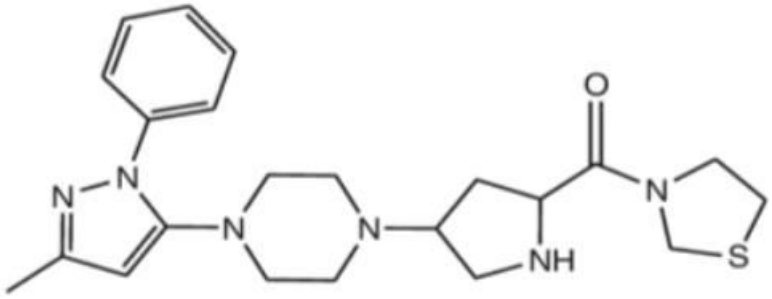

Fig.1 Chemical structure of Teneligliptin

Metformin, with the IUPAC name N,N-Dimethylimidodicarbonimidic diamide, has the chemical formula C4H11N5 (Fig. 2). It belongs to the category of anti-diabetic drugs known as Biguanides. Metformin functions by lowering blood glucose levels through several mechanisms: it reduces hepatic glucose production (gluconeogenesis), decreases the absorption of glucose from the intestines, and enhances insulin sensitivity, thereby increasing peripheral glucose uptake. These actions collectively contribute to its effectiveness in managing blood sugar levels in individuals with diabetes[2].

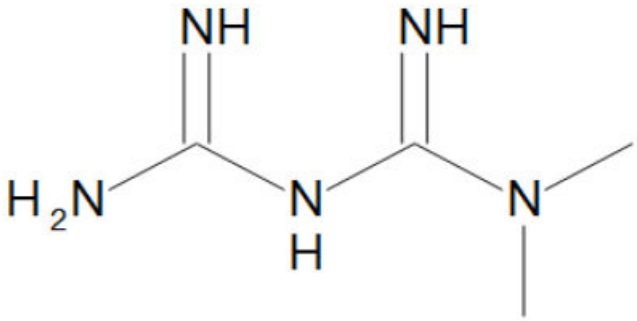

Fig.2 Chemical structure of Metformin

Pioglitazone, chemically known as 5-(4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl)thiazolidine-2,4-dione with the chemical formula C19H20N2O3S (Fig.3), belongs to the thiazolidinedione class of antidiabetic drugs. It acts as a selective agonist at peroxisome proliferator-activated receptor-gamma (PPARγ) in tissues crucial for insulin action, including adipose tissue, skeletal muscle, and liver[3].

Activation of PPARγ by pioglitazone enhances the transcription of insulin-responsive genes responsible for regulating glucose and lipid metabolism, including their production, transport, and utilization. This mechanism improves tissue sensitivity to insulin and reduces hepatic glucose production (gluconeogenesis). As a result, pioglitazone effectively addresses insulin

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resistance associated with type 2 diabetes mellitus without necessitating an increase in insulin secretion from pancreatic beta cells [4].

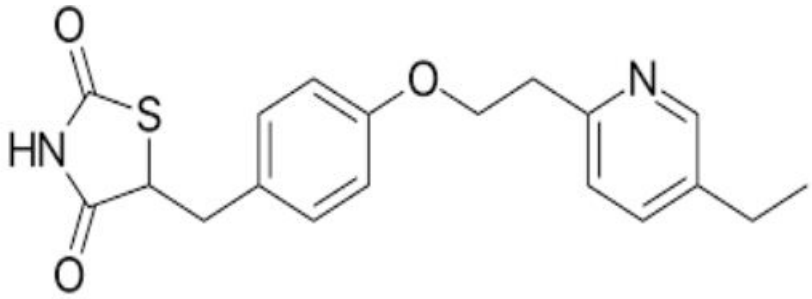

Fig.3 Chemical structure of Pioglitazone

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

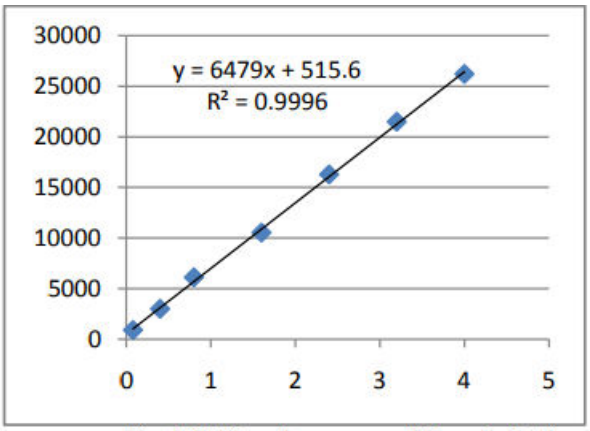

Fig.4 Calibration curve of Teneligliptin (1.6 µg/ml)

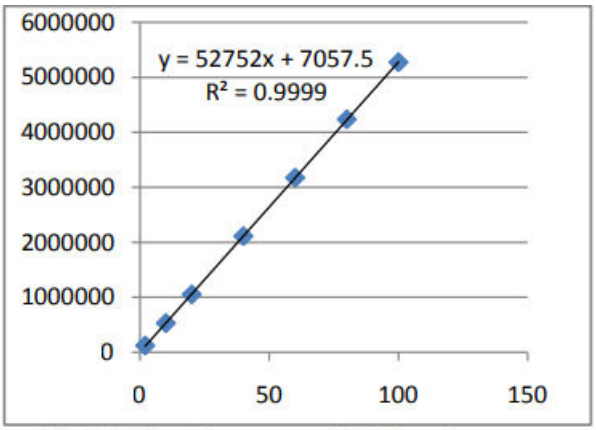

Fig.5 Calibration curve of Metformin (40 µg/ml)

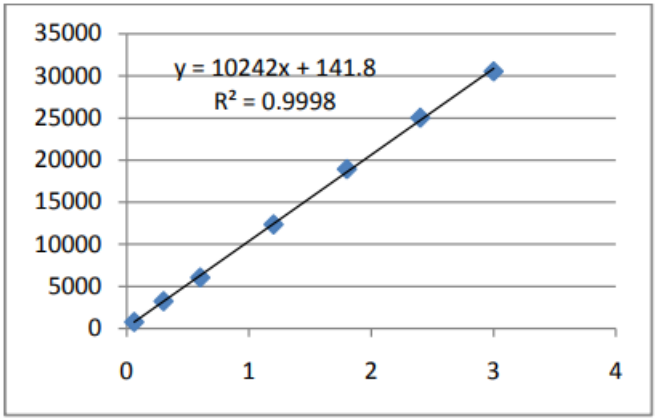

Fig.6 Calibration curve of Pioglitazone (1.2 µg/ml)

RESULTS AND DISCUSSION

The developed UPLC method involved the separation of Teneligliptin, Metformin, and Pioglitazone using a Welch C18 column (4.6 × 150 mm, 2 µm) at room temperature. The optimized mobile phase consisted 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. The retention times were 0.30 min for Teneligliptin, 0.25 min for Metformin, and 0.39 min for Pioglitazone[5].

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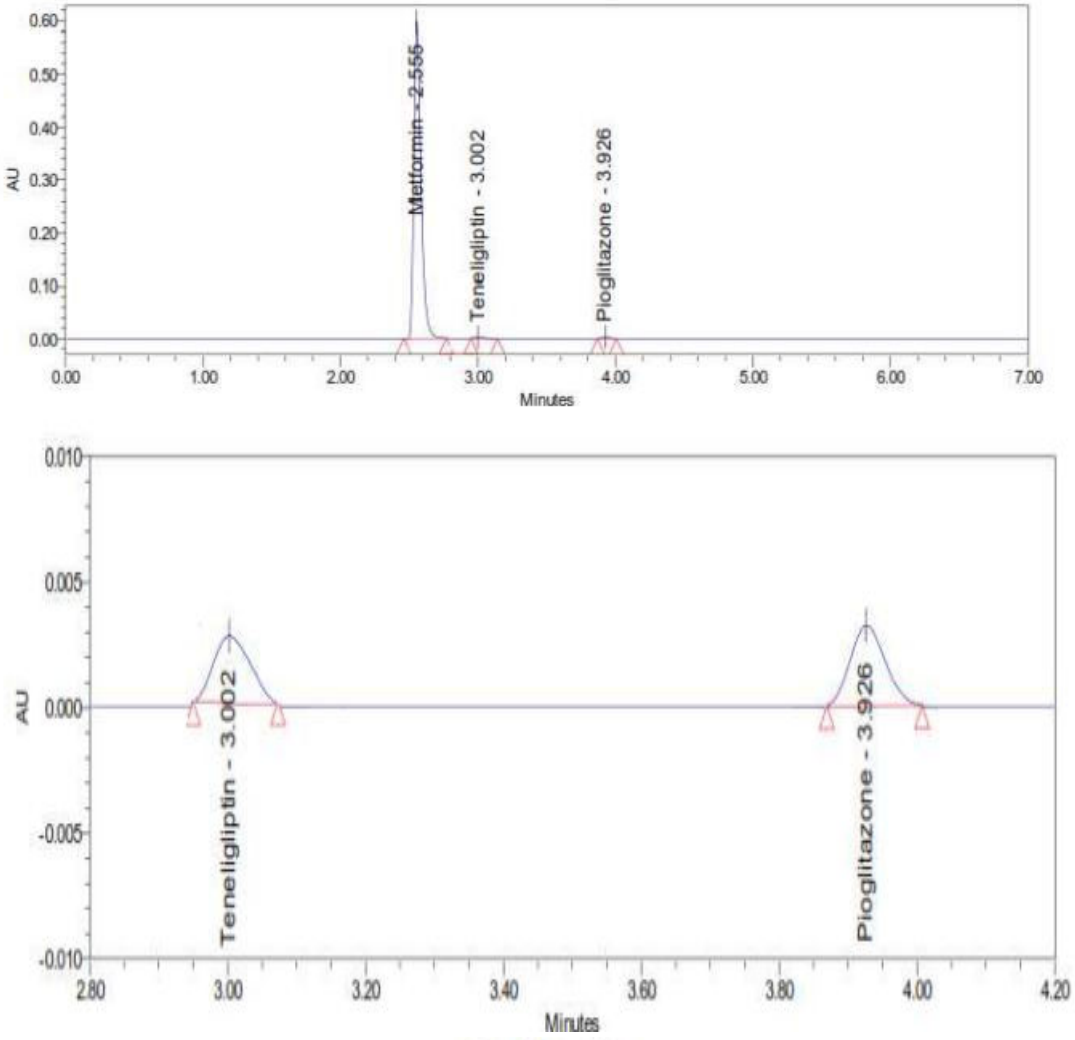
The optimized method underwent validation according to ICH guidelines. A chromatogram showing the separation of Teneligliptin, Metformin, and Pioglitazone is depicted in Fig. 7[6].



Peak Results

	Name	RT	Area	Height	Symmetry Factor	Resolution	USP Tailing	USP Plate Count	Purityt Threshold	Purityt Angle
1	Metformin	2.555	2127797	598770	1.45		1.45	12110	0.246	0.171
2	Teneligliptin	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, Teneligliptin and Pioglitazone [Methanol: ACN: Potassium Phosphate buffer (pH2.5) (70:05:25 %v/v/v].

Accuracy

The results of this study demonstrated that the method validation criteria were met, with recoveries ranging from 98.52% to 101.52% for Teneligliptin, 99.17% to 99.43% for Metformin, and 98.63% to 101.31% for Pioglitazone. These values are within the acceptable range of 98% to 102% recovery and relative standard deviations (RSD) not more than 2.0%,

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indicating the accuracy of the method for estimating Teneligliptin, Metformin, and Pioglitazone (shown in Tables 10 to 12)[6].

Limit of Detection (LOD) and Limit of Quantitation (LOQ)
The LOD values for Teneligliptin, Metformin, and Pioglitazone were determined to be 0.04 µg/ml, 0.32 µg/ml, and 0.19 µg/ml, respectively. Similarly, the LOQ values for Teneligliptin, Metformin, and Pioglitazone were found to be 0.12 µg/ml, 0.97 µg/ml, and 0.58 µg/ml, respectively (shown in Tables 13, 14)[7].

Robustness
The robustness study evaluated the method's performance under deliberate variations in parameters such as column temperature, flow rate, and pH. The assay results obtained with these variations were compared to those obtained under standard conditions, with differences not exceeding 2% as required by regulatory guidelines[8]. The obtained results were well within the acceptable limits, demonstrating that the method is robust (shown in Tables 15 to 17).

Assay
Using the UPLC method, the % assay was determined to be 100.70% for Teneligliptin, 100.50% for Metformin, and 99.98% for Pioglitazone, based on the mean of three determinations [9]. These findings indicate that the developed method is suitable for routine analysis (shown in Table 18)[10].

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 ± 44.81852	0.40
		10881		
		10957		
		10895		
		10961		
		10988		

Table 10 Recovery data for Teneligliptin

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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 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			

CONCLUSION:

Based on the observations made, it can be concluded that the validation of Teneligliptin, Metformin, and Pioglitazone in tablets using UPLC is specific, linear, accurate, precise, and robust. Therefore, the developed UPLC method is suitable for routine analysis.

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