RESEARCH ARTICLE

Development and Validation of a Stability Indicating RP-HPLC Method for Simultaneous Estimation of Metformin, Tenegliptin, and Pioglitazone in bulk and its Pharmaceutical Dosage Form

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ABSTRACT

A straightforward, reliable, and thorough approach was created for contemporaneously estimating Teneligliptin, Metformin, and Pioglitazone in solid dosage form. It was conducted by means of Agilent C18 150x4.6mm, 5µ. The mobile phase comprising buffer, acetonitrile, and Methanol (55:35:10 v/v) passed across the column at a pace of 0.9ml/min. The procedure relied on a 0.01N K2HPO4 buffer having a pH of 3.5, which was achieved by diluting an orthophosphoric acid solution. The temperature was kept constant at 30°C—optimized wavelength for Teneligliptin. Metformin and Pioglitazone were 220.0 nm. Retention times of Teneligliptin, Metformin, and Pioglitazone were 2.970 min, 2.395 min, and 3.642 min. %RSD of a system for Teneligliptin, Metformin, and Pioglitazone were 1.3, 0.6, and 1.0, respectively. %RSD of a method for Teneligliptin, Metformin, and Pioglitazone was 0.6 for each drug. The % recovery was 100.43, 100.45, and 100.22% for Teneligliptin, Metformin, and Pioglitazone, accordingly. The regression equations are used to generate the LoD and LoQ values of Teneligliptin, Metformin, and Pioglitazone, and LoD values were 0.016 ppm, 0.26 ppm, 0.42ppm, while LOQ values of Teneligliptin, Metformin and Pioglitazone were 0.048 ppm, 0.78 ppm,1.28 ppm respectively. The regression equation of Teneligliptin was y = 56655x + 920.87. Metformin was y = 14194x + 3082.1 and of Pioglitazone was y = = 41330x + 792.61.Reduced retention durations make the new approach easy and cost-effective for use in routine industrial quality control testing.

Keywords: Teneligliptin. Metformin, Pioglitazone, RP-HPLC; PDA Photodiode Array (PDA) Detection; Degradation studies; ICH Guidelines.

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Conflict of interest: None

INTRODUCTION

Metformin

Oral antidiabetic drug. It is the first-line medication of decision type 2 diabetes, specifically in overweight and fat individuals.[1,2] Depicted in Figure 1a.

Diminishes glucose creation in the liver, increments insulin awareness, and improves fringe glucose take-up. It doesn't invigorate discharge of endogenous insulin (Table 1).[3]

Pioglitazone

Is an antihyperglycemic utilized as an assistant to eat less carbs, work out, and other antidiabetic meds to overse. particular agonist at peroxisome proliferator-enacted receptor-gamma (PPARγ)[4] tabulated in Table 2, and Figure 1b.

Teneligliptin

Treatment of Type 2. Sodium-glucose co-transporter-2 (SGLT-2) inhibitor[5-8] depicted in Table 3, Figure 1c.

MATERIAL AND METHODS

Instrumentation

The water-HPLC 2695 system, which includes an auto-injector and a PDA detector, was utilized. Empower 2 is the main program utilized. Lab India's UV-VIS spectrophotometer The absorbance of solutions containing Metformin, Pioglitazone, and Teneligliptin was measured using a UVwin5 instrument with a 2 mm and 10 mm specialized bandwidth and matched quartz.

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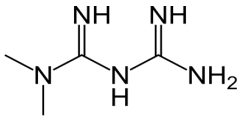

Figure 1a: Chemical structure of Metformin

Table 1: Metformin

Synonym:	N,N-Dimethylimidodicarbonimidic Diamide-d6, Hydrochloride; Diabetosan-d6; Diabex-d6, Metformin-d6; Metiguanide-d6
Application:	A deuterium labeled oral hypoglycemic agent
Molecular Weight:	171.66
Molecular Formula:	$C_4H_6D_6ClN_5$
Appearance:	Crystalline
Physical State:	Solid
Solubility:	Soluble in DMSO, and methanol.
Storage:	Store at -20° C
Melting Point:	215-218°C (lit.)

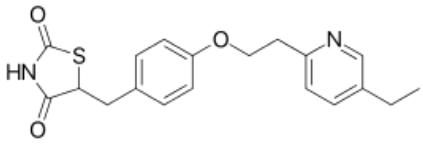

Figure 1b: Chemical structure of Pioglitazone

Table 2: Pioglitazone

CAS Number	: 111025-46-8
Purity	: ≥99%
Molecular Weight	: Average: 356.439
Molecular Formula	: $C_{19}H_{20}N_2O_3S$
Physical State	: Solid
Pka	: 6.66(Strongest acid) , 5.6(Base)
Solubility methanol, ethanol	: Practically insoluble in water, Soluble in DMF,
Melting Point	: 193-194 °C (lit.) [23-25]

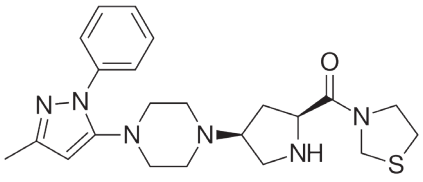

Figure 1c: Chemical structure of Teneliglipin

Chemicals

Metformin, Teneligliptin, and Pioglitazonereference standard was extracted from Zita pio met (200 + 20 + 15) label claim Metformin 200 mg Teneligliptin 20 mg Pioglitazone15mg per unit formulation was obtained from Glenmark Labs Panaji. The solvent employed in the trial was acetonitrile.

Table 3: Teneligliptin

CAS Number	: 864070-44-0
Purity	: 98%
Molecular Weight	: 450.91
Molecular Formula	: C23H27ClO7
Appearance	: White powder
Physical State	: Solid
Solubility	: It is very slightly soluble in water, soluble in methanol ethanol and acetonitrile; practically insoluble in toluene.
Storage	: 20-25° C

Chromatographic Condition

All of the glassware that was used in the method was immersed in a solution of sulfuric acid and chromic acid overnight, washed well using double-distilled water, and then dried using a hot air oven. Agilent C18 Column (150mm x 4.6 mm, 5 µm) was used at 30 °C. Isocratic elution was performed withBuffer, Acetonitrile, and Methanol in the ratio of (55:35:10) v/vmixture. The flow rate was 0.9 ml/min, and PhotoDiodearray detection was at 220 nm. Metformin, pioglitazone, and teneligliptin eluted within 5 minutes.[9,10]

Preparation of Standard Stock Solutions

Three 100 ml volumetric jars were used for the separate transfer of 5 mg of [Tene], 250 mg of [Met], and 3.75 mg of [Pio]. A total of 20 minutes after adding 10 milliliters of diluent, the flagons were combined. Flasks were filled with (2500 µg/ml or ppm of Metformin, 50 µg/mL or ppm of Teneligliptin, 37.5 µg/mL or ppm of Pioglitazone).[11,12]

Method Validation

In order to ensure that the proposed approach is linear, accurate, precise, and robust, it was tested according to the ICH Q2 (R1) standards.[13]

Robustness

Although subtle adjustments were made to the procedure, such as the flow rate, mobile phase ratio, and temperature, no discernible difference was seen in the outcome, and everything remained within the parameters specified by the ICH guidelines.

Accuracy

Three degrees of recovery experiments were conducted to evaluate the accuracy of the suggested method: 50%, 100%, and 150%. Three different concentrations of a reference drug solution were added to the pre-analyzed sample solution, and the absorbance readings were taken. Afterward, the formula was used to determine the percentage recovery.

% Recovery = A – B/ C

Specifically, where A is the overall drug quantity calculated, B is the quantity of drug detected on a pre-analyzed basis, and C is the proportion of pure drug provided.

RESULTS AND DISCUSSION

Screening and Optimization

Selection of the detection wavelength

Figure 2 depicts the UV spectra of Metformin, Pioglitazone, and Tenegliptin in the ratio of 55:35:10 v/v mixtures of Buffer, acetonitrile, and Methanol in the region between 200 and 400 nm. This signifies that at 220.0 nm, Metformin, Pioglitazone, and Tenegliptin have maximum absorbance. Therefore, the optimal detection wavelength for the quantitation was determined to be the maximum concentration of Metformin, Pioglitazone, and Tenegliptin in the mobile phase.[14]

Calibration of standards

Metformin, pioglitazone, and teneligliptin were all calibrated using the standard curve. Various concentrations of 250, 5, and 3.75 µg/ml solutions were produced by properly transferring different quantities of every stock solution into 10 mL volumetric flasks and diluting them to the mark. The calibration line was derived by comparing the peak area to the drug concentration.[15,16]

Method development

Optimized chromatographic conditions are shown in Figure 3

- Column: Agilent C18 Column (150 mm x 4.6 mm, 5 µm).
- Mobile phase: Buffer and Acetonitrile and Methanol (55:35:10)
- Flow rate: 0.9 mL/min
- Detector: PDA 220 nm
- Temperature: 30°C
- Injection Volume: 10 µL

System suitability

Following the protocol, a five-time injection of a Molnupiravir standard solution was administered into the HPLC apparatus

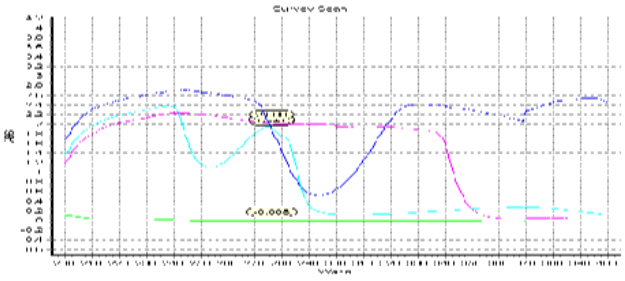

Figure 2: Absorbance spectrum metformin, pioglitazone and tenegliptin

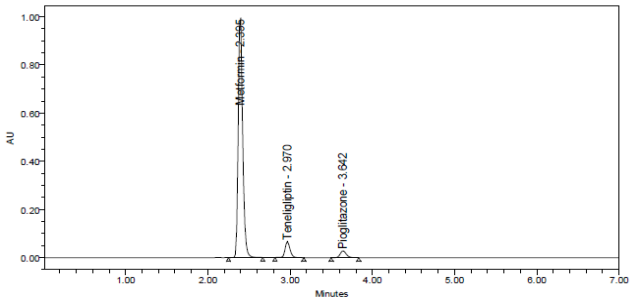

Figure 3: Optimized chromatographic condition

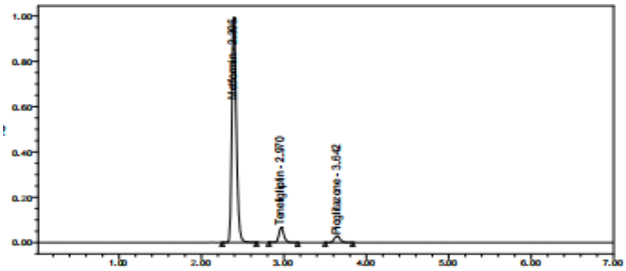

Figure 4: System suitability

shown in Figure 4. The system suitability characteristics were assessed using standard chromatograms by determining the percent relative standard deviation of retention time, tailing factor, theoretical plates, and peak areas from five duplicate injections. All of these values fell within the specified range; results are tabulated in Table 4.

Linearity

Within a certain range, it is the method's capacity to provide test results that are proportionate to the analytic concentration. It is often shown as the variation of the slope or regression line. A 10 mL volumetric flask was used to transfer the accurate volume of the standard stock solution. A solution with 10-60 ppm was obtained by adjusting the volume of the mobile phase to reach the mark. Appropriate calculations were made for the slope, Y-intercept, and correlation coefficient. Through the use of regression analysis, a regression line was developed that connected the standard medication concentrations. Table 5 displays the findings of the regression analysis, while Figures 5-7 show the linear calibration curves within the range of the study.

Table 4: System suitability data

S. No	*Metformin*			*Teneligliptin.*				*Pioglitazone*			
Inj	*RT (min)*	*TP*	*Tailing*	*RT (min)*	*TP*	*Tailing*	*RS*	*RT (min)*	*TP*	*Tailing*	*RS*
1	2.364	8983	1.2	2.919	11134	1.16	5.2	3.340	10679	1.18	3.4
2	2.365	8936	1.21	2.92	10676	1.16	5.1	3.347	10551	1.17	3.4
3	2.365	8955	1.2	2.922	10367	1.17	5.2	3.347	10482	1.16	3.4
4	2.365	8912	1.2	2.922	10335	1.18	5.2	3.349	10271	1.16	3.4

Table 5: Linearity data

Teneligliptin.		*Metformin*		*Pioglitazone*	
Conc (µg/mL)	*Peak area*	*Conc (µg/mL)*	*Peak area*	*Conc (µg/mL)*	*Peak area*
1.25	70870	62.5	884965	0.93	38537
2.5	94479	125	1775921	1.8	77303
3.75	143703	187.5	2657571	2.81	117229
5	189271	250	3591894	3.75	157372
6.25	237860	312.5	4429674	4.68	189706

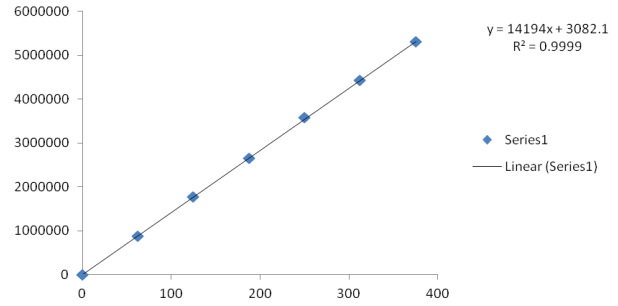

Figure 5: Calibration curve of metformin

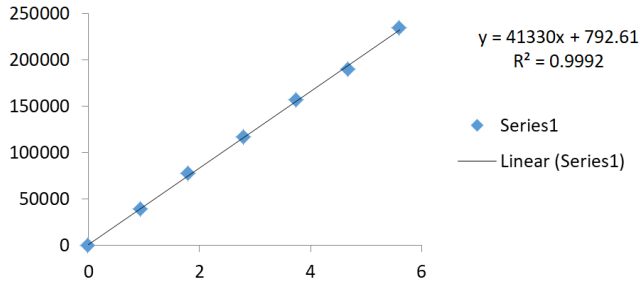

Figure 6: Calibration curve of pioglitazone

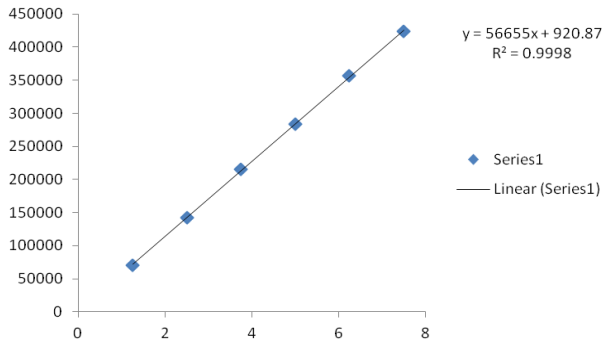

Figure 7: Calibration curve of teneligliptin

Precision

Six working sample solutions of 40 ppm are injected, and the % Amount found was

On the day after preparing samples, six 40 ppm working sample solutions were injected; the following day, the dosage was estimated, and the %RSD was determined to be between the prescribed ranges, indicating intermediate precision. Standard

Table 6: Precision data

S. No	*Area of teneligliptin*	*Area of metformin*	*Area of pioglitazone*
1.	281038	3528265	155718
2.	285925	3585690	156290
3.	288566	3546093	157164
4.	279599	3556708	155729
5.	281875	3531610	154251
6.	279036	3526017	152876
Mean	282673	3545731	155338
S.D	3776.0	22849.3	1535.0
%RSD	1.3	0.6	1.0

Table 7: Accuracy data of Metformin

% Level	*Amount spiked (µg/mL)*	*Amount recovered (µg/mL)*	*% Recovery*	*Mean %Recovery*
	125	124.8	99.8	
50%	125	125.7	100.6	
	125	125.5	100.4	
	250	250.9	100.3	
100%	250	250.4	100.2	100.45%
	250	251.9	100.8	
	375	377.4	100.7	
150%	375	377.1	100.6	
	375	378.1	100.8	

Table 8: Accuracy data of Teneligliptin

%Level	*Amount Spiked (µg/mL)*	*Amount recovered (µg/mL)*	*% Recovery*	*Mean %Recovery*
	2.5	2.52	100.85	
50%	2.5	2.52	100.84	
	2.5	2.52	100.60	
	5	5.01	100.26	
100%	5	5.02	100.36	100.43%
	5	4.98	99.68	
	7.5	7.55	100.68	
150%	7.5	7.53	100.34	
	7.5	7.52	100.24	

Requirements: No surpassing two percent should be the relative standard deviation (RSD) for any of the five standard injections results are shown in Table 6

Accuracy

Three different concentrations were injected three times: 50, 100, and 150%. The percentage of recovery was then determined as 100.45, 100.45, and 100.22% .results are shown in Table 7-9.

Table 9: Accuracy data of Pioglitazone

%Level	*Amount Spiked (µg/mL)*	*Amount recovered (µg/mL)*	*% Recovery*	*Mean %Recovery*
50%	1.8	1.83	101.90	100.22%
	1.8	1.80	99.99	
	1.8	1.80	99.92	
100%	3.75	3.76	100.33	
	3.75	3.80	101.21	
	3.75	3.74	99.86	
150%	5.6	5.59	99.90	
	5.6	5.58	99.70	
	5.6	5.55	99.20	

Robustness

Methods are intentionally tweaked in small ways, for example, by varying flows, mobile phases, temperature, and mobile phase plus or minus. We compute the %RSD of the criteria above.

LoD and LoQ

The formulas for calculating LOD and LOQ are 3.3 x(σ/S) and 10 x (σ/S), accordingly. At this point σ represents the standard deviation of the intercept and S is the average of the slope. S/N may also be used to calculate the LOQ and LoD.

A number of 3-5 is appropriate for LoD and 10-15 for LoQ.

Assay of marketed formulation

Separate injections of the standard and sample solutions were made into the system; chromatograms are presented in Figure 8, 9, and Table 10 shows the results of the drug concentration calculation performed using the method mentioned earlier.

Degradation studies

The formulation was subjected to degradation experiments, and injection samples of the degraded substance were collected. Tables 11, 12, and 13 show the findings of the assay conducted on the injected samples, which demonstrated that all of the samples reached the degradation thresholds.

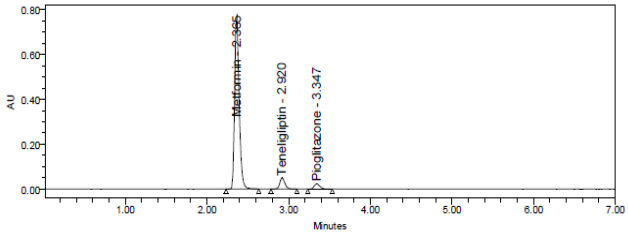

Figure 8: Chromatogram of working standard solution

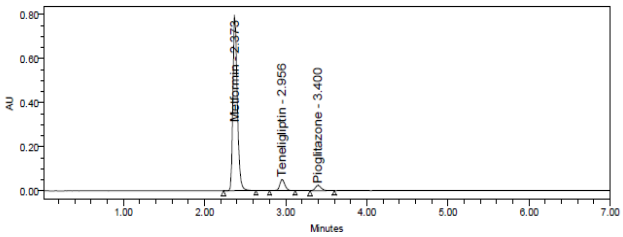

Figure 9: Chromatogram of working sample solution

Degradation Procedure

Oxidation

Hydrogen peroxide (H2O2), a dissolving agent, was integrated directly into 1 mL of molnupiravir stock solution. The mixtures were heated to 60 °C for 30 minutes. To conduct the HPLC investigation, 10 µL of the diluted solution was introduced into the device, and its chromatograms were tracked to evaluate the sample's stability, shown in Figure 10

Acid degradation studies

Stir 1 milliliter of molnupiravir stock solution with 1 milliliter of 2 N HCl. Reflux for 30 minutes at 1°C. To prepare for the HPLC investigation, 10µl of the diluted solution was introduced into the equipment, and its chromatograms were acquired to evaluate the sample's stability, as shown in Figure 11.

Alkali degradation studies

Stir 1 milliliter of molnupiravir stock solution with 1 milliliter of 2 N sodium hydroxide. Reflux for 30 minutes at 60°C. To prepare for the HPLC investigation, 10 µL of the diluted solution was introduced into the equipment and its

Table 10: Assay of marketed

S. No	*Standard Area*	*Sample area*	*% Assayof Tenegliptin*	*% Assay of metformin*	*% Assay Pioglitazone*
1	281038	280994	99.21	100.82	99.17
2	285925	282128	99.61	100.28	100.64
3	288566	284682	100.51	99.29	99.84
4	279599	285729	100.88	99.58	99.1
5	281875	284351	100.39	100.49	100.42
6	279036	283901	100.23	99.67	99.86
Avg	282673	283631	100.14	100.02	99.84
Stdev	3776	1749.9	0.62	0.596	0.628
%RSD	1.3	0.6	0.6	0.6	0.6

Table 11: Degradation Data of Metformin

S. No	*Degradation condition*	*% Drug undegraded*	*% Drug degraded*	*Peak areas*
1	Acid	97.83	2.17	274386
2	Alkali	98.05	1.95	276089
3	Oxidation	94.37	5.63	264828
4	Thermal	98.08	1.92	278898
5	UV	98.30	1.70	279930
6	Water	99.15	0.85	281838

Table 12: Degradation Data of Teneligliptin

S. No	*Degradation condition*	*% Drug undegraded*	*% Drug degraded*	*Peak areas*
1	Acid	96.87	3.13	274386
2	Alkali	97.48	2.52	276089
3	Oxidation	93.50	6.50	264828
4	Thermal	98.47	1.53	278898
5	UV	98.83	1.17	279930
6	Water	99.51	0.49	281838

Table 13: Degradation Data of Pioglitazone

S. No	*Degradation condition*	*% Drug undegraded*	*% Drug Degraded*	*Peak Areas*
1	Acid	95.35	4.65	148412
2	Alkali	95.97	4.03	149380
3	Oxidation	94.46	5.54	147022
4	Thermal	96.87	3.13	150778
5	UV	97.22	2.78	151316
6	Water	98.86	1.14	153879

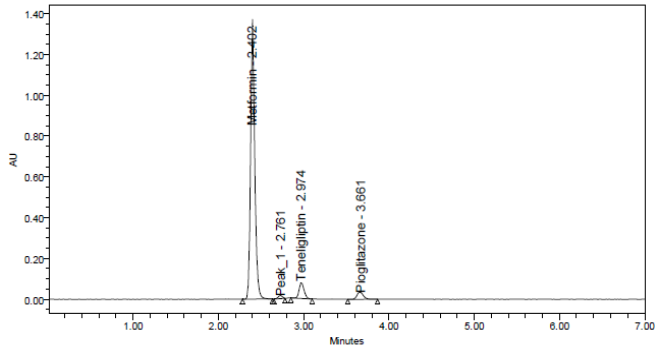

Figure 10: Peroxide degradation chromatogram

chromatograms were acquired to evaluate the sample's stability (Figure 12).

Dry heat degradation studies

The deterioration of the standard medication solution due to dry conditions was studied by placing it in an oven at 1050c for 6 hours. To prepare for the HPLC investigation, 10µl of the diluted solution was introduced into the equipment and

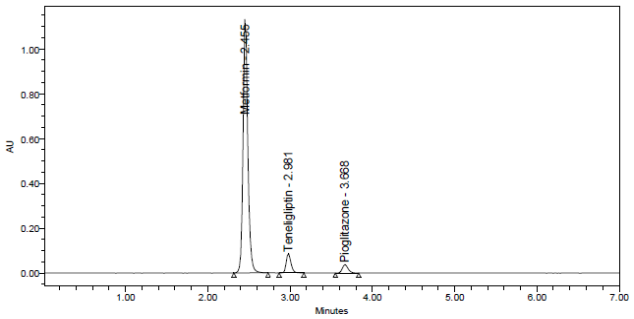

Figure 11: Acid degradation chromatogram

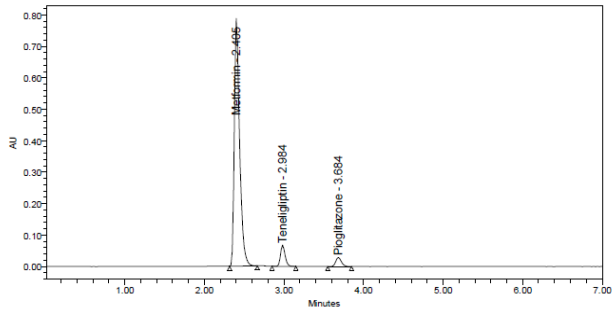

Figure 12: Base degradation chromatogram

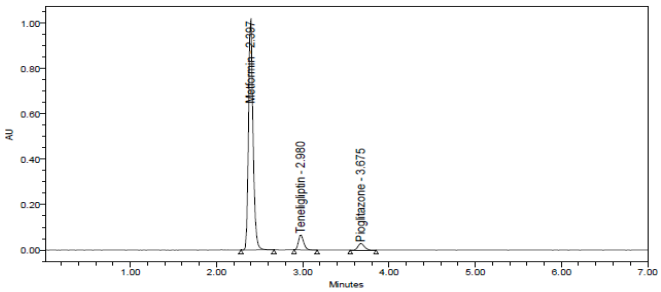

Figure 13: Thermal degradation chromatogram

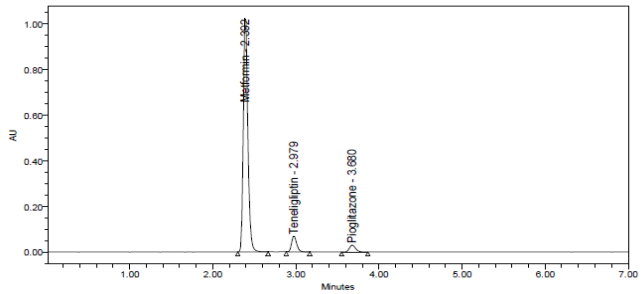

Figure 14: UV degradation chromatogram

its chromatograms were acquired to evaluate the sample's stability (Figure 13).

Photostability studies

The drug's photochemical stability was further investigated by subjecting the 400 ppm sample to ultraviolet light for seven days in a UV chamber or 200 Wh/m² in a photostability cell. To prepare for the HPLC investigation, 10 µL of the diluted solution was introduced into the

Table 14: Summarized data of teneligliptin, metformin and pioglitazone

Parameters		*Teneligliptin.*	*Metformin*	*Pioglitazone*	*LIMIT*
Linearity Range(µg/mL)		50-300	62.5-375	6.25-37.5	
Regression coefficient		0.999	0.999	0.999	
Slope(m)		56655	14194	41330	R< 1
Intercept(c)		920.87	3082.1	792.61	
Regression equation (Y=mx+c)		y = 56655x + 920.87	y = 14194x + 3082.1	y = 41330x + 792.61	
Assay (% mean assay)		100.14%	100.02%	99.84%	90-110%
Specificity		Specific	Specific	Specific	No interference
System precision %RSD		1.3	0.6	1.0	NMT 2.0%
Method precision %RSD		0.6	0.6	0.6	NMT 2.0%
Accuracy % recovery		100.43%	100.45%	100.22%	98-102%
LOD (µg/ml)		0.016	0.26	0.012	NMT 3
LOQ (µg/ml)		0.048	0.78	0.036	NMT 10
Robustness	FM	0.3	0.4	0.6	%RSD NMT 2.0
	FP	0.3	1.5	1.4	
	MM	0.6	0.5	1.2	
	MP	1.2	1.0	1.3	
	TM	0.4	1.0	1.2	
	TP	1.0	0.4	1.7	

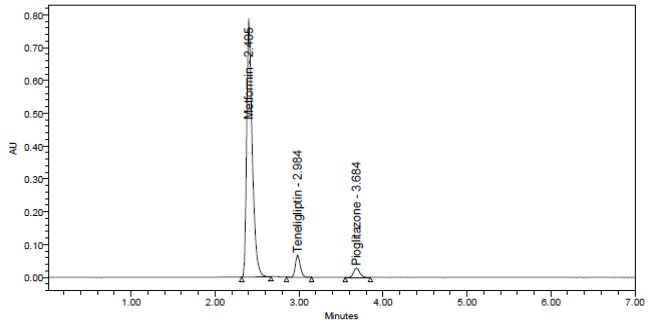

Figure 15: Water degradation chromatogram

equipment, and its chromatograms were acquired to evaluate the sample's stability, as shown in Figure 14. The final solution had a concentration of 40 ppm.

Neutral degradation studies

The medication was refluxed with water at 60ºC for 6 hours to study stress testing in neutral circumstances. To conduct the HPLC investigation, 10µl of the diluted solution was introduced into the device, and its chromatograms were tracked to evaluate the sample's stability, as shown in Figure 15. The final solution had a concentration of 40 ppm.

DISCUSSION

Research on acid-base degradation has shown that adjusting the mobile phase pH affects the retention duration of pharmaceuticals. However, since the acidic sample was neutralized using a 2N base solution and the base sample was neutralized using a 2N acid solution, the retention period would remain the same.

CONCLUSION

A straightforward, reliable, and thorough approach was created for contemporaneously estimating Teneligliptin, Metformin, and Pioglitazone in Tablet dosage form. Retention times of Teneligliptin, Metformin, and Pioglitazone are 2.970, 2.395, and 3.642 minutes. %RSD of system precision for Teneligliptin, Metformin, and Pioglitazone were 1.3, 0.6, and 1.0 correspondingly. %RSD of a method for Teneligliptin, Metformin, and Pioglitazone was 0.6 each. % recoveries were 100.43%, 100.45% and 100.22% for Teneligliptin, Metformin and Pioglitazone correspondingly. The regression equations are used to generate the LoD and LoQ values of Teneligliptin, Metformin, and Pioglitazone, and LoD values were 0.016, 0.26, and 0.42ppm, while LoQ values of Teneligliptin, Metformin and Pioglitazone were 0.048, 0.78, and 1.28 ppm respectively. The regression equation of Teneligliptin was y = 56655x + 920.87. Metformin was y = 14194x + 3082.1 and of Pioglitazone was y = = 41330x + 792.61. Reduced retention durations make the new approach easy and cost-effective for use in routine industrial quality control testing. Summarised data of metformin, teneligliptin, and pioglitazone were found, and the results are shown in Table 14.

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ETHICS

Ethics Committee Approval

The ethics committee approval not required for the proposed research. We were not used any kind of human being and animal matrices.

Informed Consent

Not applicable.

Authorship Contributions Concept

GKS, Design: GKS, Data Collection or Processing: MB, Analysis or Interpretation:MB.
Literature Search: MB. Writing: GKS.

AUTHOR'S CONTRIBUTIONS

All authors contributed equally in the study. They made substantial contributions to the design of the study, the collection of the data as well as the preparation and analysis of the data. They also drafted the manuscript and gave final approval for its submission to the journal for consideration of publication.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported. Also, this research did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sector. It was funded by personal efforts of the authors.

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