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An Effective and Time-Saving HPTLC Densitometry Technique for the Measuring of Antidiabetic Triple-Drug Combination: From TLC Spot Formation to Method Validation

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Abstract. A precise, rapid, linear & robust High-Performance Thin-Layer Chromatography (HPTLC)–Densitometric was successfully developed and validated method for the simultaneous estimation of a triple antidiabetic drug combination comprising Metformin Hydrochloride (MET), Teneligliptin Hydrobromide Hydrate (TEN), and Pioglitazone Hydrochloride (PIO) in bulk and ph'ceutical formulation. Chromatographic separation was achieved using precoated silica gel 60 F254 TLC plates with a mobile phase made up of n-butanol, 1,4-dioxane, glacial acetic acid, and n-hexane in a ratio of 4:2:2:2 (%v/v/v/v). Densitometry was used for detection at 242 nanometers. Spots were obtained using distinct and well-resolved Rf values 0.20 ± 0.01, 0.55 ± 0.02, and 0.90 ± 0.01 for MET, TEN, and PIO, respectively. The ICH Q2 (R1) guidelines were followed in the validation of the approach, demonstrating excellent linearity over the ranges of concentrations of 1000–3500 nanogram/band for MET, 40–140 nanogram/band for TEN & 30–115 nanogram/band for PIO, with correlation coefficients (r^2) of 0.9983, 0.9961, and 0.9965, respectively. The recovery studies confirmed the method's accuracy, yielding mean recoveries of 99.92% (MET), 100.03% (TEN) & 100.97% (PIO). The LOD and LOQ were determined to be 154.22 nanogram/band and 467.34 nanogram/band for MET, 8.66 nanogram/band and 26.25 nanogram/band for TEN, and 10.95 ng/spot and 33.18 ng/spot for PIO, respectivelyThe assay results further confirmed the reliability of the developed method, showing percentage recoveries of 99.03% (MET), 99.34% (TEN), and 99.03% (PIO). Overall, the proposed HPTLC–densitometric method provides a simple, economical and effective analytical tool for the routine quality control and simultaneous quantification of Metformin hydrochloride, Teneligliptin hydrobromide hydrate, and Pioglitazone

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1 Introduction

HPTLC or densitometry method is a highly advanced instrumental technique that builds upon the principles of traditional thin layer chromatography (TLC). It plays a crucial role in the profiling of pharmaceutical and natural compounds, As well as contemporary drugs, throughout various stages of drug discovery and development [1]. HPTLC-densitometry offers significant advantages, including automation, precise scanning, complete optimization, and a selective detection mechanism. These features, along with minimal sample preparation and the potential for hyphenation with other techniques, make HPTLC an indispensable tool for obtaining chromatographic data on complex mixtures found in pharmaceuticals, natural products, clinical samples, food substances, and more [2]. Diabetes mellitus (DM) is a condition that affects metabolism, characterized by high blood sugar levels. It encompasses different forms, including type 1, type 2, non-insulin-dependent diabetes with early onset, pregnancy-induced diabetes, congenital diabetes mellitus, and diabetic condition that results from other factors, such as hormonal disorders or the use of steroids [3]. A combination of TEN, MET and PIO can be particularly effective for managing type 2 diabetes. Each drug in this combination works differently to control blood sugar: Metformin hydrochloride**:** 3-(diamino methylidene)-1,1-dimethylguanidine hydrochloride [Figure 1_A] is a biguanide that lowers liver glucose production and enhances insulin sensitivity in muscles and fat, without increasing insulin secretion [4].

Teneligliptin hydrobromide hydrate**:** {[(2S,4S)-4-[4-(3-methyl-1-phenyl-1H-pyrazol-5-yl) piperazin-1-yl] pyrrolidin-2-yl] methyl} thiazole hydrobromide hydrate [Figure 1_B] is a DPP-4 inhibitor that increases incretin hormones, promoting insulin release in response to high blood sugar, especially after meals [5].

Pioglitazone hydrochloride: 5-[[4-[2-(5-ethyl-2-pyridinyl) ethoxy] phenyl] methyl]-1,3-thiazolidine-2,4-dione hydrochloride [Figure 1_C] Activates PPAR-γ, improving insulin sensitivity in muscles and fat, and influencing genes involved in glucose and lipid metabolism [6].

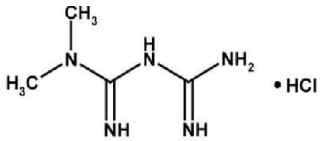

Figure 1A: Chemical structure of Metformin hydrochloride

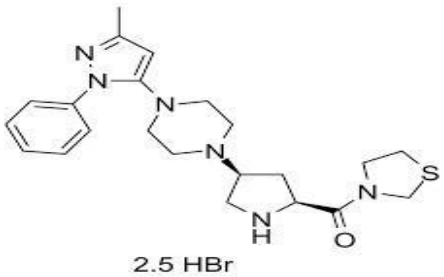

Figure 1B: Chemical Structure of teneligliptin hydrobromide hydrate

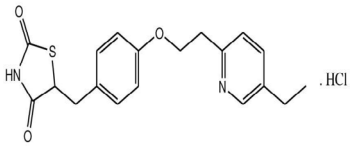

Figure 1C: Chemical structure of pioglitazone hydrochloride

Together, these drugs address different aspects of diabetes by reducing insulin resistance, enhancing insulin secretion, and decreasing glucose production, offering a comprehensive approach to managing the disease [7]. A literature review revealed that several methods, including HPLC, HPTLC, UV-spectrophotometry, and spectrofluorometric, have been reported for the analysis of MET, TEN and PIO, either individually or in combination with other drugs [8-25]. However, these methods are complex and rely on expensive instruments and chemicals, making them unsuitable for small-scale laboratories. As a result, the decision was created to create and verify a cost-effective & straightforward densitometry method for analysis.

2. Experimental work

2.1 Materials

2.1.1 Chemicals and reagents:

The materials required for the advancement of TLC include ultra-pure solvents like n-Butanol, n-Hexane, glacial acetic acid, and 1,4-Dioxane. Pure drug samples of metformin HCl, teneligliptin HBr hydrate, and pioglitazone HCl were procured from Dhamtec Pharma and Consultants, located in Navi Mumbai, Maharashtra, India.

The marketed formulations were obtained from a local pharmacy. Each tablet, as per the label claim, contained 500 milligrams of MET, 20 milligrams of TEN, and 15 milligrams of PIO.

2.1.2. Instrumentation:

The research incorporated a densitometric system (Camag linemate) equipped with a partially automated sample applicator (Linemate V), a 100 microliters Hamilton syringe, a HPTLC densitometer (CAMAG) IV, and Camag WinCATS software. Additional equipment included a 20 × 10-centimeter CAMAG Twin-Trough thin layer chromatographic chamber, dual UV lamp viewing Chamber, a Shimadzu AUX-220 electronic analytical balance, and a Toshcon ultra-sonicator (model 4.5).

2.2 Method

2.2.1 Development of TLC:

The active pharmaceutical ingredients MET, TEN, and PIO were individually dissolved in methanol to prepare stock solutions at concentrations of 5000 ppm, 300 ppm, and 150 ppm, respectively, ensuring that the applied volume of 0.2 µL per band corresponded to the desired amount per spot for densitometric analysis. The mobile phase used for TLC development was a mixture of n-Butanol, 1,4-Dioxane, Glacial Acetic Acid,

and n-Hexane in the ratio 4:2:2:2 (% v/v/v/v), which was optimized after preliminary trials to achieve clear and well-resolved separation of the three drugs on silica gel 60 F_{254} plates. In this system, n-Butanol and 1,4-Dioxane provided suitable polarity to facilitate migration of moderately polar compounds, Glacial Acetic Acid improved peak shape and minimized tailing, and n-Hexane adjusted the overall polarity to enhance resolution between closely migrating bands. Thin-layer chromatograms were developed for the individual APIs, as shown in Figure 2_A & for the marketed formulation in combination with the APIs, as presented in Figure 2_B.

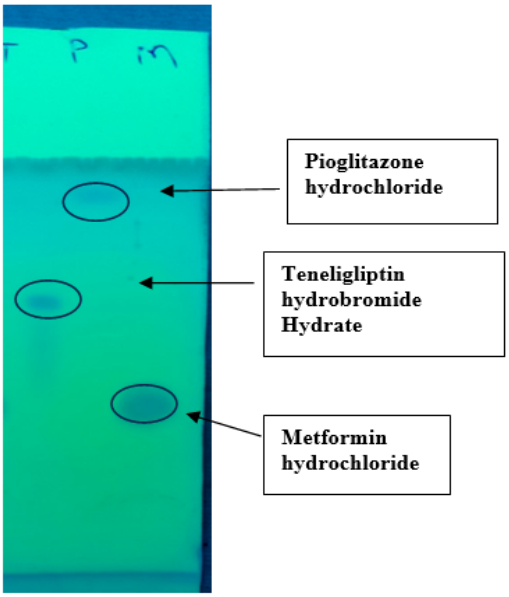

Figure 2A: TLC of API

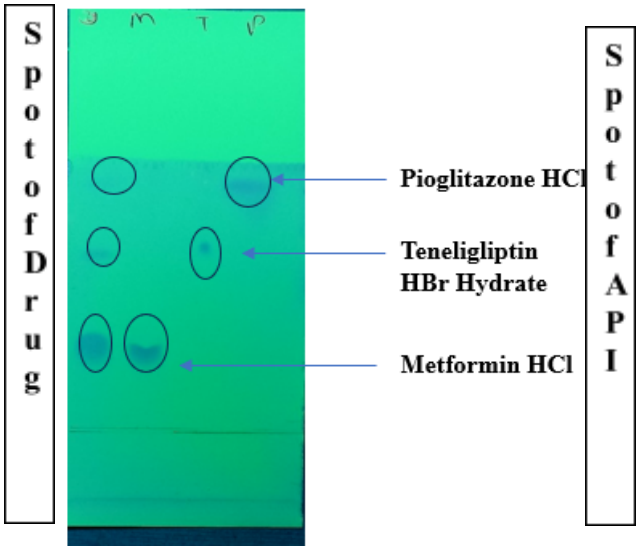

Figure 2B: TLC of API + marketed formulation (drug)

2.2.2 Chromatographic Condition:

Aluminium-backed Silica Gel 60 F_{254} TLC plates (20 × 10 cm, Merck, Germany) were pre-washed with methanol and activated at 60°C for 5 minutes. The mobile phase consisted of n-Butanol, 1,4-Dioxane, Glacial Acetic Acid, and n-Hexane in a 4:2:2:2 (% v/v/v/v) ratio. Samples were applied as 6.0 mm wide bands using a CAMAG Linemate 5 semi-automatic applicator at 8.0 mm from the lower edge with 15 mm centre-to-centre spacing, a Predose volume of 0.2 µL per band, a dispensing speed of 150 nL s^{-1}, and a 100 µL syringe, applying a total of 18 tracks per plate. The amount per spot ranged from 1–3.5 µg for MET, 0.04–0.14 µg for TEN, and 0.03–0.115 µg for PIO. Plates were developed in a CAMAG dual-trough chamber pre-saturated with the mobile phase for 30 minutes at room temperature to a distance of 90 mm, air-dried, and scanned densitometrically using a Camag TLC Image Acquisition System IV over 200–400 nm with a 4.00 × 0.30 mm slit and a scanning rate of 20 mm s^{-1}, showing optimal response at 242 nm (Figure 3_A), and the standard chromatogram is presented in Figure 3_B.

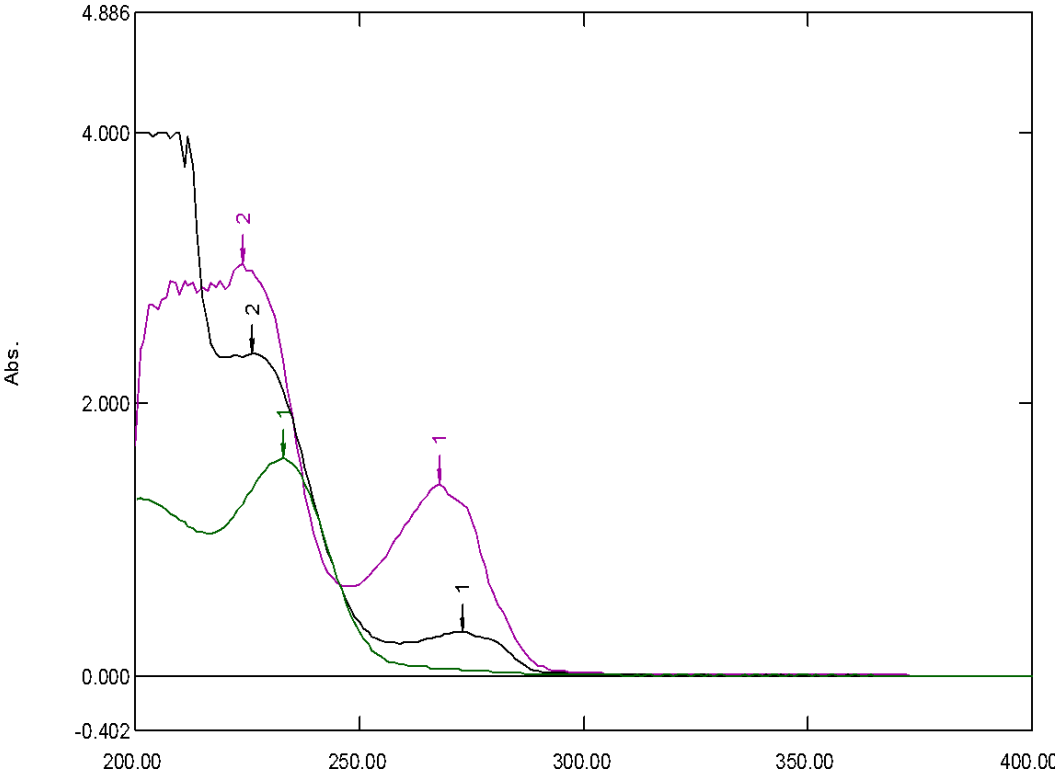

Figure 3A: Wavelength Selection for MET, TEN & PIO

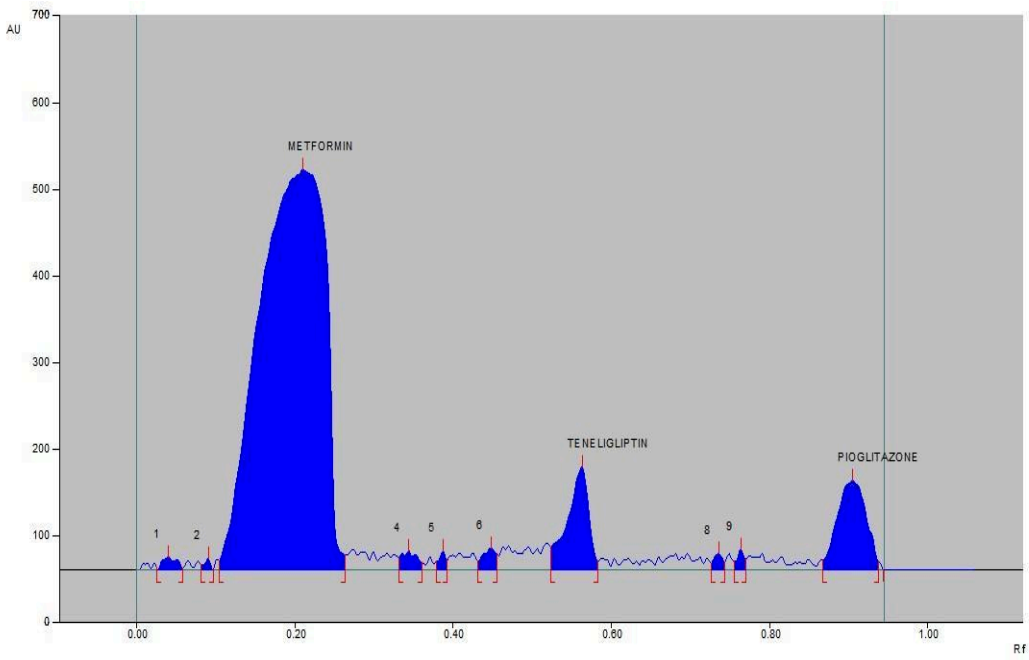

Figure 3B: Standard chromatogram

2.3 Calibration stock solution preparation:

The Preparation of the calibration stock solution involved accurately weighing 50 milligrams of Metformin Hydrochloride (MET), 2 mg of Teneligliptin Hydrobromide Hydrate (TEN), and 1.5 mg of Pioglitazone Hydrochloride (PIO). The measured quantities of the pure drugs were transferred into the analyte was dispersed in a limited amount of methanol in a 10 mL measuring flask. After complete dissolution, It was filled up to the mark at 10 mL by using methanol. This resulted in final stock concentrations of 5000 micrograms per millilitre for MET, 200 micrograms per millilitre for TEN, and 150 micrograms per millilitre for PIO.

2.4 Preparation of test solutions:

20 tablets containing a labelled amount of 500 mg Metformin Hydrochloride (MET), 20 mg 15 mg of pioglitazone hydrochloride (PIO) and teneligliptin hydrobromide hydrate (TEN) were precisely weighed and ground into a fine powder. One tablet's worth of powder, which contained 500 milligrams of MET, 20 milligrams of TEN, and 15 milligrams of PIO, was weighed and put into a 100-millilitre volumetric flask. A few drops of methanol were added to dissolve the sample & the solution was subsequently sonicated for 20 minutes to ensure complete extraction of the active components. After sonication, the mixture was brought up to the desired volume 100 mL with methanol to obtain the sample stock solution. Taken from this solution, 3 millilitre was pipetted into a 10-millilitre calibrated flask and the volume was adjusted by using methanol to achieve the final concentrations of 1500 Micrograms per millilitre for Metformin HCl, 60 Micrograms per millilitre for Teneligliptin HBr hydrate & 45 Micrograms per millilitre for Pioglitazone.

3. Validation of analytical procedure

Performance assessment of the developed techniques was conducted in agreement with the validation parameters outlined by the ICH Q2(R1) guidelines [24].

3.1 Linearity and range:

The proportionality of the results was examined by evaluating five independent standardization points within the ranges of 1000 – 3500 ng/spot for MET, 40 – 140 ng/spot for TEN, and 30 – 115 ng/spot for PIO. Quantitative standard plots were constructed by graphing area under the peak respect to the corresponding concentrations, and the R-value along with the regression line equation was calculated.

3.2 Accuracy:

Accuracy in a chemical analysis method refers to representing the extent of similarity between the measured value and benchmark value. Spike the sample solutions with known quantities of the standard solution at distinct concentration levels (e.g., 80%, 100%, and 120%) of the nominal concentration. This involves adding the known standard solution to the formulation matrix must make sure the matrix doesn't obstruct the detection of the analyte. Recovery % must be between 98% - 102%. All the solutions were spotted and analyzed as described under chromatographic conditions.

3.3 Intermediate Precision:

Precision reflects the consistency of results when a method is repeatedly applied to multiple portions of a homogeneous sample under specified conditions. Intermediate precision measures variability within a laboratory, such as differences between days, analysts, or instruments

3.1.1 Intra-day precision: Day-to-day consistency of the proposed method was examined using of the proposed method was assessed by applying 2, 3, and 4 microliters of the calibration standard solution onto the thin layer chromatographic plate three times within the same day and analysing it across the full concentration range under the specified chromatographic conditions.
3.1.2. Inter-day precision: It was determined by applying 2, 3, and 4 microliters of the calibration standard solution onto TLC plates three times on different days and analysing it over the entire concentration range as outlined in the chromatographic conditions.

3.4 Lowest detectable and quantifiable concentrations

3.4.1 Limit of detection (LOD): Predicated on visual assessment visual evaluation can be applied to instrumental as well as non-instrumental procedures. It is the minimum concentration of the analyte that the method can reliably detect within the matrix, though not quantify, with a specified level of confidence.

$$\mathbf{LOD = 3.3\sigma / S}$$

3.4.2 Limit of quantification (LOQ): Predicated on Visual Assessment visual evaluation can be applied to instrumental as well as non-instrumental procedures. Denotes the lowest

detectable concentration of the analyte with exactness and consistency with acceptable levels of reliability.

LOQ=10σ / S

Where σ = Standard deviation

S= Slope

3.5 Robustness:

Robustness refers to the competence of a method to remain stable despite minimum, intentional modifications in Variables. To assess the robustness of the method, variations made in chromatographic scanning speed (18 millimetres per second, 20 millimetres per second, and 22 millimetres per second) and the solvent composition.

4. Assay for marketed formulation (combination of MET, TEN and PIO):

A sample solution is prepared according to section 2.6. From this, 3 mL was diluted with methanol to 10 mL in order to achieve assay concen. of 1500 microgram/millilitre for MET, 60 microgram/millilitre for TEN, and 45 microgram/millilitre for PIO. The resulting solution was introduced onto the chromatographic plate and analysed using the validated HPTLC method. The assay was conducted in three replicates, and the amount of drug was estimated through the respective calibration curves.

5. Result & Discussion

5.1 Linearity

A robust linear association was observed in the calibration plots over the selected concentration intervals for each drug: 1000-3500 ng/band for MET, 40-140 ng/spot for TEN, and 30-115 ng/band for PIO, as depicted in Figure 4. The regression equations and coefficient of determination (r^2) values, summarized in Table 1, confirmed excellent linearity. Specifically, MET showed an r^2 value of 0.9983 (Figure 5), TEN had an r^2 value of 0.9961 (Figure 6), and PIO exhibited an r^2 value of 0.9985 (Figure 7). UV image of Linearity for all three drugs is given in a Figure 8.

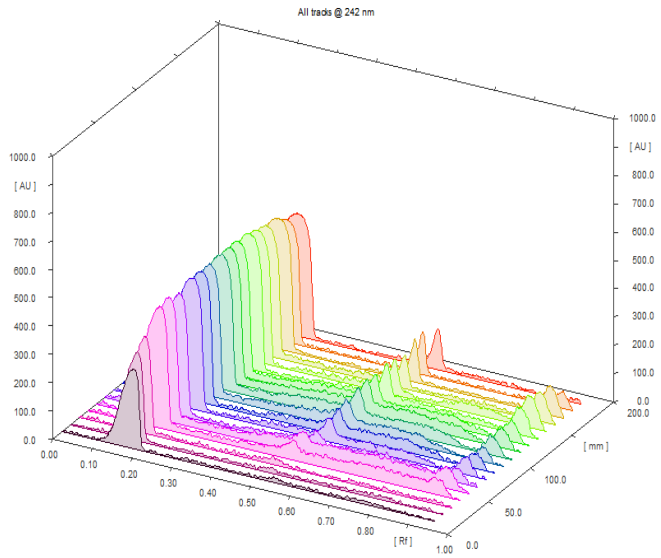

Figure 4: 3D chromatogram of Linearity for metformin hydrochloride, teneligliptin hydrobromide hydrate & pioglitazone hydrochloride

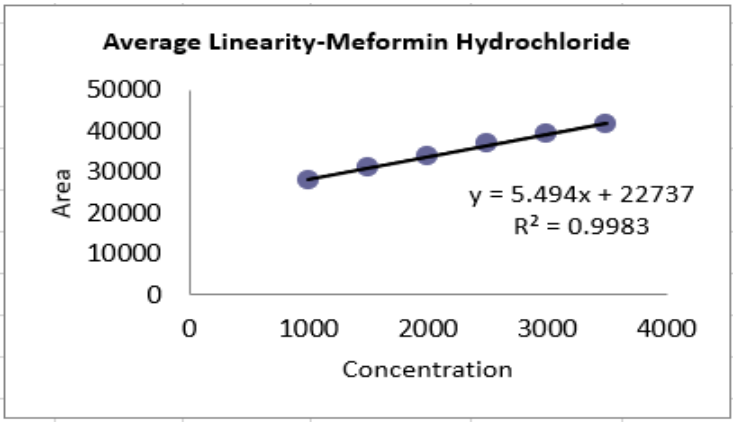

Figure 5: Calibration curve of metformin HCl

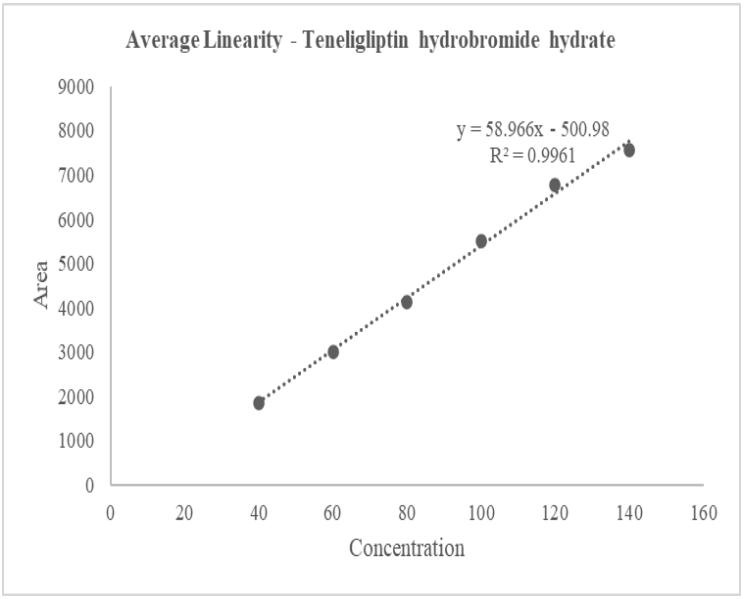

Figure 6: Calibration Curve of Teneligliptin hydrobromide hydrate

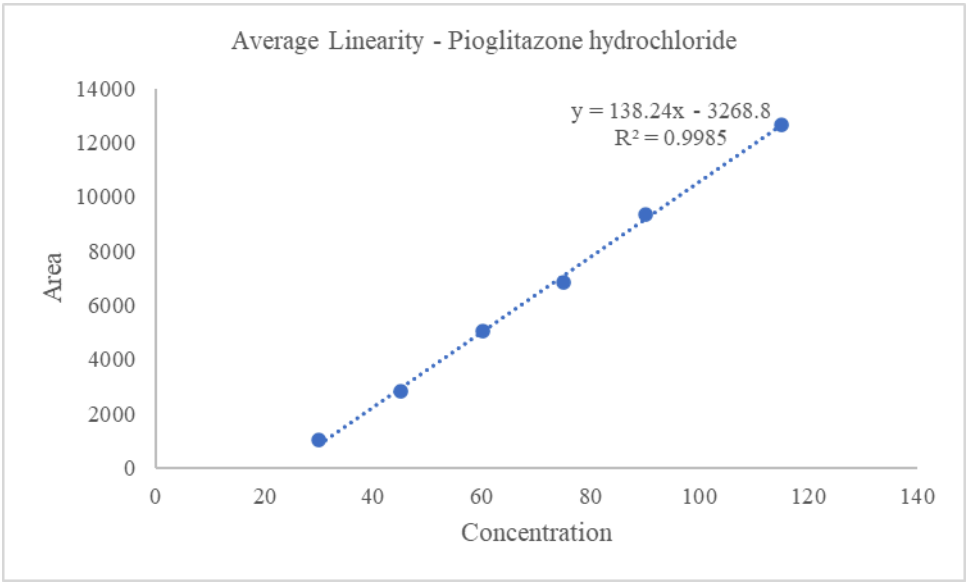

Figure 7: Calibration curve of pioglitazone hydrochloride

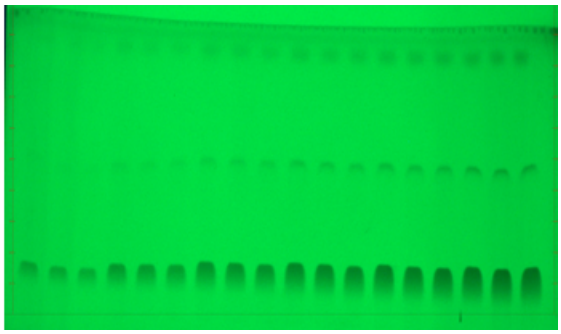

Figure 8: Linearity of Metformin HCl, Teneligliptin HBr Hydrate & Pioglitazone HCl

Table 1. Linearity & Range for Metformin HCl, Teneligliptin HBr Hydrate & Pioglitazone HCl

Parameter	Metformin hydrochloride	Teneligliptin hydrobromide hydrate	Pioglitazone hydrochloride
Concentration Range	1000-3500 ng/spot	40-140 ng/spot	30-115 ng/spot
Slope	5.49	58.96	138.24
Intercept	22737	- 500.98	-3268.8
r^2	0.9983	0.9961	0.9985
LOD	154.22 ng/spot	8.66 ng/spot	10.95 ng/spot
LOQ	467.34 ng/spot	26.25 ng/spot	33.18 ng/spot

5.2 Accuracy:

The recovery findings were found to be inside the allowable limits established by ICH Q2(R1) recommendations. The range of recovery percentages for MET was 99% to 101%, for TEN it was 99% to 102% & for PIO was 99% to 102%. As shown in Table 2, these values were calculated for formulation samples as well as pure drug samples at 80%, 100%, and 120% levels. Detailed result is given in a Table 2.

Table 2. Accuracy for metformin HCl, teneligliptin HBr hydrate and pioglitazone HCl

Drug	Level (%)	Amount of sample (ng/spot)	Amount of std. spiked (ng/spot)	Total amount (ng/spot)	% Recovery (avg.)
Metformin hydrochloride	80	1500	1200	2700	100.14
	100		1500	3000	99.84
	120		1800	3300	99.78
Teneligliptin hydrobromide hydrate	80	60	48	108	99.46
	100		60	120	101.11
	120		72	132	99.50
Pioglitazone hydrochloride	80	45	36	81	101.75
	100		45	90	101.66
	120		54	99	99.50

5.3 Precision

The method's consistency was evaluated based on its repeatability. For MET, TEN and PIO intra-day and inter-day variations were analysed at three different concentration levels:

1000, 1500 and 2000 ng/band for MET; 40, 60, and 80 ng/spot for TEN; and 30, 45, and 60 ng/band for PIO. The %RSD values for all concentration levels were found to be below 2%, demonstrating excellent precision. Detailed results are presented in Table 3.

Table 3. Intraday & Interday Precision study of metformin Hydrochloride, teneligliptin hydrobromide hydrate and pioglitazone hydrochloride

Drug Name	Conc. (ng/spot)	Day	Average Peak area	S.D.	%RSD	Day	Average Peak area	S.D.	%RSD
		Intraday Precision				Interday Precision			
Metformin hydrochloride	1000	**1 (Morning)**	28761.72	177.95	0.61	**1**	28761.72	177.95	0.61
	1500	**1 (Afternoon)**	32148.60	188.17	0.58	**2**	32148.60	188.17	0.58
	2000	**1 (Evening)**	32506.89	183.38	0.56	**3**	32506.89	183.38	0.56
Teneligliptin hydrobromide hydrate	40	**1 (Morning)**	1833.97	15.94	0.86	**1**	1833.97	15.94	0.86
	60	**1 (Afternoon)**	2924.04	26.89	0.91	**2**	2924.04	26.89	0.91
	80	**1 (Evening)**	4112.37	40.60	0.98	**3**	4112.37	40.60	0.98
Pioglitazone hydrochloride	30	**1 (Morning)**	1165.07	8.71	0.74	**1**	1165.07	8.71	0.74
	45	**1 (Afternoon)**	2837.23	18.08	0.63	**2**	2837.23	18.08	0.63
	60	**1 (Evening)**	5178.34	25.86	0.49	**3**	5178.34	25.86	0.49

5.4 LOD & LOQ

Minimum detectable concentration & Minimum quantifiable concentration for MET were 154.22 nanogram/band and 467.3 nanogram/band, respectively. For TEN, minimum detectable concentration & minimum quantifiable concentration were 8.66 nanogram/band and 26.25 nanogram/band, sequentially. For PIO, minimum detectable concentration & minimum quantifiable concentration were 10.95 nanogram/band and 33.18 nanogram/band, sequentially. A summary of these results can be found in Table 1.

5.5 Robustness

The RSD % of method less than 2% was obtained by calculating the S.D. value of peak areas for each experimented parameters, as shown in Table 4**.**

Table 4: Robustness for metformin HCl, teneligliptin HBr hydrate and pioglitazone HCl

Parameter	Condition	Metformin Hydrochloride		Teneligliptin hydrobromide hydrate		Pioglitazone hydrochloride	
		%RSD	Rf	%RSD	Rf	%RSD	Rf
Unaltered		1.20	0.20	1.04	0.55	1.27	0.90
Change in Mobile phase Composition	4.1:1.9:2:2	1.23	0.18	1.03	0.545	1.27	0.91
	4:2:2.1:1.9	1.20	0.19	1.04	0.55	1.27	0.90
Change in a Chromatographic scanning speed	18 mm/s	1.21	0.20	1.04	0.55	1.26	0.90
	22 mm/s	1.20	0.20	1.03	0.55	1.27	0.90

5.6 Assay of MET, TEN and PIO:

Table 5 displays the results of the quantification of the marketed tablet. After analysis, the percentages of MET, TEN and PIO were found to be 99.03%, 99.34% and 99.03%, respectively. UV image for comparison of Test with Standard is given in a Figure 9 as for reference.

Table 5: Assay of metformin HCl, teneligliptin HBr hydrate and pioglitazone HCl

Drug	Label claim	Amount obtained %	Assay
Metformin hydrochloride	500 mg	495.15 mg	99.03%
Teneligliptin hydrobromide hydrate	20 mg	19.86 mg	99.34%
Pioglitazone hydrochloride	15 mg	14.85 mg	99.03%

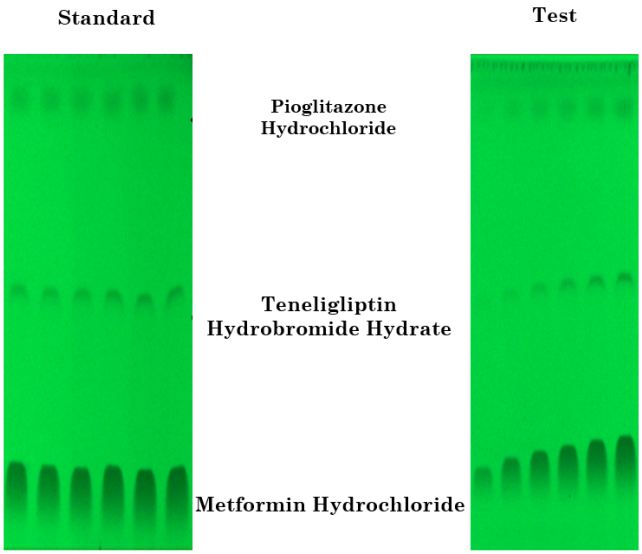

Figure 9: UV image for comparison of Test with Standard

6. Highlights and Merits of the Present Method

The present HPTLC method for the simultaneous estimation of MET, TEN, and PIO demonstrates excellent linearity, precision, and accuracy, fully complying with ICH Q2(R1) validation guidelines. It provides superior separation with clearly resolved Rf values—MET (0.20), TEN (0.55), and PIO (0.90)—ensuring enhanced peak resolution and reliable identification. The method offers notable sensitivity, particularly for TEN and PIO, as reflected by lower detection and quantification limits compared to previously published techniques. In addition, it avoids reliance on complex statistical software or advanced experimental designs, making it user-friendly and well-suited for both academic laboratories and routine quality control environments. Specifically developed for fixed-dose combination marketed tablets, the method demonstrates practical relevance for formulation analysis and routine quality assessment. Compared to previously reported HPTLC methods for MET, TEN, and PIO, this approach provides improved resolution, employs a simpler mobile phase composition, and achieves comparable or lower limits of detection, highlighting its robustness and suitability for routine quality control of the triple drug combination.

7. Conclusion

The concurrent analysis of Metformin Hydrochloride (MET), Teneligliptin HBr Hydrate (TEN), and Pioglitazone HCl (PIO) in a fixed-dose pharmaceutical formulation was effectively accomplished using a validated HPTLC-densitometric method. The developed method exhibited distinct and well-resolved Rf values for each analyte, ensuring reliable chromatographic separation with minimal interference. Comprehensive Method verification, carried out as per the ICH Q2(R1) standards, verified the procedure's linear behaviour, exactness, repeatability, selectivity, and detection capability. The high correlation coefficients and excellent recovery values underscore the applied procedure's

analytical consistency under varied conditions, reproducibility & suitability for conventional quality analysis. Owing to its operational simplicity, reduced analysis time, and cost-effectiveness, The developed High Performance thin layer chromatographic method offers a reliable and efficient analytical approach provides a scientifically sound and practical alternative for the regular quantification of MET, TEN, and PIO in complex antidiabetic formulations.

8. List of Abbreviation

MET – Metformin hydrochloride**, PIO –** Pioglitazone hydrochloride**, TEN –** Teneligliptin hydrobromide hydrate**,** GAA – Glacial acetic acid**, API –** Active pharmaceutical ingredient**, TLC –** Thin layer chromatography**, HPTLC –** High performance thin layer chromatography

9. Conflict of Interest: The authors have no conflicts of interest.

References

1. Shewiyo DH, Kaale E, Risha PG, Dejaegher B, Smeyers-Verbeke J, Vander Heyden Y. HPTLC methods to assay active ingredients in pharmaceutical formulations: A review of the method development and validation steps. J Pharm Biomed Anal. 2012; 66:11–2.
2. Sonia K, Bhavya Shree B, Lakshmi KS. HPTLC method development and validation: An overview. J Pharm Sci Res. 2017;9(5):652–7.
3. Prajapati P, Kapupara P, Vekariya H. Simultaneous analysis of remogliflozin etabonate and vildagliptin in tablets by HPTLC: Method development and validation. Res J Pharm Technol. 2024;17(12):5697–5701.
4. Metformin [Internet]. Available from: https://go.drugbank.com/drugs/DB00331
5. Teneligliptin [Internet]. Available from: https://go.drugbank.com/drugs/DB11950
6. Pioglitazone [Internet]. Available from: https://go.drugbank.com/drugs/DB01132
7. Jamwal N. Systematic review on medicaments used in management of diabetes mellitus. Int J Pharm Pharm Sci. 2020;12(8):1–5.
8. International Conference on Harmonization (ICH). Validation of Analytical Procedures: Text and Methodology Q2(R1).
9. Pandya S, Vekaria HJ. Analytical methods for estimating metformin hydrochloride, pioglitazone, and teneligliptin in diabetes management: A comprehensive review. J Chromatogr Sci. 2025;63(3):1–9.
10. Vekaria H, Limbasiya V, Patel P. Development and validation of RP-HPLC method for simultaneous estimation of Montelukast Sodium and Fexofenadine hydrochloride in combined dosage form. J Pharm Res. 2013;6:134–9.
11. Patel BN, Vekaria HJ. Forced degradation studies and development of RP-HPLC method for related substances of Tenofovir alafenamide in tablet dosage form. Anal Chem Lett. 2023;13(3):321–36.
12. Mishra K, Soni H, Nayak G, Patel SS, Singhai AK. Method Development and Validation of Metformin Hydrochloride in Tablet Dosage Form. E-J Chem. 2011;8(3):1309–13.
13. Arayne MS, Sultana N, Zuberi MH. Development and Validation of RP-HPLC Method for the Analysis of Metformin. Pak J Pharm Sci. 2006;19(3):231–5.
14. Sen AK, Hinsu DN, Sen DB, Zanwar AS, Maheshwari RA, Chandrakar VR, et al. Analytical method development and validation for simultaneous estimation of Teneligliptin hydrobromide hydrate and Metformin hydrochloride from its pharmaceutical dosage form by three different UV spectrophotometric methods. J Appl Pharm Sci. 2016;6(09):157–65.

15. Vetapalem R, Yejella RP, Atmakuri LR. Development and validation of a stability indicating RP-HPLC method for simultaneous estimation of teneligliptin and metformin. Turk J Pharm Sci. 2020;17(2):141–8.
16. Gadapa N, Tripathi UM. RP-HPLC analytical method development and validation for simultaneous estimation of three drugs: glimepiride, pioglitazone, and metformin and its pharmaceutical dosage forms. J Chem. 2013;2013:1–8.
17. Singh D, Dwivedi SC, Kushnoor A. Development and validation of a HPTLC method for estimation of pioglitazone in bulk and tablet dosage form. J Pharm Res. 2011;4(11):3919–21.
18. Dubey S, Kashyap P, Pandey A, Thakur D. A validated method development for estimation of pioglitazone by first-order derivative spectroscopy. Int J PharmTech Res. 2013;5(4):1451–5.
19. Dharmamoorthy G, Nandini Y, Anupama M, Sai Nandhini M, Jhansi Grace Susmitha K, Kesava Reddy Y. Development and validation of stability indicating HPTLC method for the simultaneous estimation of metformin hydrochloride and pioglitazone in pharmaceutical dosage form. YMER. 2022;21(8):612–26.
20. Sengupta P, Bhaumik U, Ghosh A, Sarkar AK, Chatterjee B, Bose A, et al. LC–MS–MS development and validation for simultaneous quantitation of metformin, glimepiride, and pioglitazone in human plasma and its application to a bioequivalence study. J Chromatogr B. 2009;69:1243–50.
21. Kusuma Kumari G, Krishnamurthy PT, Ammu RVVV, Vishwanath K, Narenderan ST, Babu B, et al. Development and validation of a sensitive LC-MS/MS method for pioglitazone: application towards pharmacokinetic and tissue distribution study in rats. RSC Adv. 2021;11:11437–43.
22. Modi DK, Patel BH. Rapid and sensitive simultaneous estimation of metformin hydrochloride and pioglitazone hydrochloride in tablet formulation by HPTLC method. J Liq Chromatogr Relat Technol. 2013;36(5):618–27.
23. Patel BD, Vekaria HJ. Central Composite Design Expert-Supported RP-HPLC Optimization and Quantitative Evaluation of Efonidipine Hydrochloride Ethanolate and Chlorthalidone in Tablet. J Chromatogr Sci. 2024;62(6):585–92.
24. Patel BN, Vekaria HJ. A stability indicating reverse phase high performance liquid chromatography method for related substances of Sofosbuvir in tablet dosage form. Int J Pharm Sci Drug Res. 2023;15(05):547–55.