Development and Validation of HPTLC method for the simultaneous estimation of Teneligliptin hydrobromide hydrate and Pioglitazone hydrochloride using a DOE approach

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ABSTRACT

A high-performance thin layer chromatographic (HPTLC) method was developed for simultaneous quantification of Teneligliptin hydrobromide hydrate (TNG) and Pioglitazone hydrochloride (PIO) using design of experiment methodology. Separation was achieved on silica gel 60 F254 aluminium plates with a mobile phase of Toluene: Methanol: Ethyl acetate: Ammonia (5.5:2.3:2.2:0.05, v/v/v/v) and detection at 231 nm. Robustness testing employed a fractional factorial design (2^{4-1}) considering mobile phase composition (A), solvent front (B), wavelength (C), and chamber saturation time (D). The solvent front significantly affected the Rf values of both drugs, thus requiring careful control. Rf values were 0.561 for TNG and 0.789 for PIO. Linearity was established over 2000–12000 ng/band for TNG and 1500–9000 ng/band for PIO. Recovery rates ranged from 99.94–100.90% for TNG and 99.13–100.12% for PIO. The method was validated for linearity, precision, accuracy, and specificity following ICH Q2(R1) guidelines, with %RSD below 2% for both analytes. This accurate, reproducible, and simple HPTLC method is suitable for routine quality control of pharmaceutical formulations.

KEYWORDS: Teneligliptin, Pioglitazone, Development and validation, Design of experiment, High Performance thin layer chromatography

INTRODUCTION

Type 2 diabetes, sometimes referred as noninsulin dependent diabetes. Teneligliptin and pioglitazone are the recommended secondline treatments for type 2 diabetes. Teneligliptin has a distinctive peptidomimetic architecture built from five successive ring structures.[1] In the Xray cocrystal of teneligliptin with DPP4, the phenyl ring attached to the pyrazole moiety interacts intimately with the S2 extended subsite of DPP4, a binding that is believed to contribute substantially to the drug's high potency and selectivity.[2,3]

Teneligliptin works by blocking the enzyme DPP4, which slows down the degradation of the hormones GLP1 and GIP.[4] This causes their levels to remain higher, and as a result insulin release is enhanced, glucagon secretion is suppressed, gastric emptying is delayed, and blood sugar levels fall.[5]

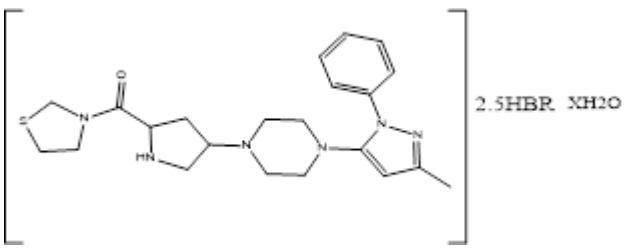


Figure 1: Chemical structure of Teneligliptin.

Pioglitazone (also known chemically as **(RS)5(4[2(5ethylpyridin2yl) ethoxy] benzyl) thiazolidine2, 4dione**) is an oral hypoglycaemic drug belonging to the thiazolidinedione class.[6]

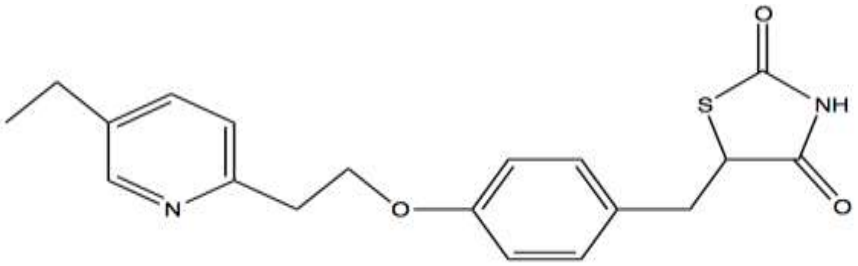


Figure 2: Chemical structure of Pioglitazone.

Thiazolidinediones act as potent ligands for peroxisome proliferator activated receptor gamma (PPARγ), a type of ligandactivated nuclear receptor. Upon activation by a ligand, PPARγ pairs with the retinoid X receptor (RXR) to form a heterodimer [7,8]. This complex binds to specific DNA sequences to regulate the transcription of genes that control glucose and lipid metabolism. Various PPARγ agonists—such as rosiglitazone, pioglitazone, and troglitazone—differ in how they influence the expression of over a hundred PPARγresponsive genes [9,10]. These differences likely arise from variations in receptor conformations and coactivator protein interactions. Activation of PPARγ by pioglitazone enhances insulin sensitivity in the liver, adipose tissue, and peripheral tissues. Additionally, its active metabolites—MII and MIV (hydroxy derivatives) and MIII (a keto derivative)—help improve glucose regulation by decreasing insulin resistance [11].

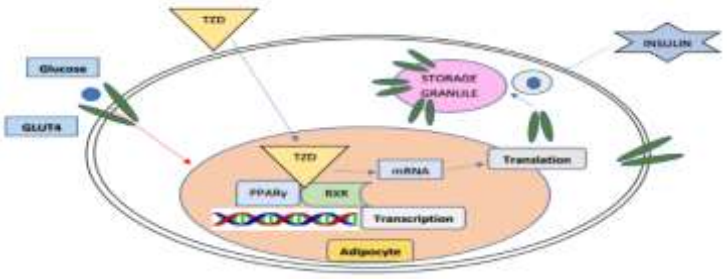


Fig 3: Mechanism of action Pioglitazone.

Literature evidence indicates that various analytical methods, including RPHPLC and spectrophotometric techniques, have been reported for the individual and simultaneous estimation of TNG and PIO. [12-49] Despite several analytical methods being available, there is no report on an HPTLC technique for the concurrent estimation of TNG and PIO in combination dosage form. The growing interest in HPTLC stems from its advantages, including cost effectiveness, rapid analysis, low solvent consumption, minimal sample cleanup, and suitability for automated sample application and detection.

In this study, the robustness of the HPTLC analytical method was investigated using a fractional factorial design (FFD). This response surface approach was preferred over conventional designs because it allows efficient prediction of nonlinear responses, requires fewer experimental runs, and provides comprehensive information regarding the main and interaction effects of influential factors. Consequently, a novel, precise, simple, and

reproducible HPTLC method was developed for the simultaneous estimation of TNG and PIO in a pharmaceutical dosage form, with robustness evaluated using a fractional factorial design (FFD). Furthermore, this research describes the creation and validation of an HPTLC method, using a Design of Experiments (DoE) framework, to simultaneously quantify teneligliptin (TNG) and pioglitazone (PIO).

MATERIALS AND METHODS

Reference standards of teneligliptin (TNG) and pioglitazone (PIO) were kindly provided as complimentary samples by Precise Chemipharma pvt ltd., Mumbai, and Abhilasha Pharma pvtltd, Ankleswar. All reagents and solvents used throughout the study were of analytical grade and sourced from Merck Specialities pvtltd., India. The commercially available tablet formulation evaluated in this work—produced by Glenmark Pharmaceuticals Ltd.—was procured from the local market.

a) Instrumentation

The experimental work was conducted using a Linomat 5 applicator, TLC Scanner IV, and UV chamber (Camag, Switzerland) controlled via Vision CATS software version 3 (Camag, Switzerland). Sample application was performed using a Linomat syringe (659.0014, Hamilton Bonaduz Schweiz, Camag, Switzerland), and chromatographic development was achieved in a twin trough chamber (20 *10 cm). coated silica gel 60 F_{254} aluminium plates (20 *10 cm, 100 µm thickness; Merck, Darmstadt, Germany) were used as the stationary phase.

b) Preparation of standard solutions

Stock solutions of TNG and PIO (1000 µg/mL) were prepared individually by accurately weighing 10 mg of each drug, dissolving in methanol, and diluting to 10 mL with the same solvent in volumetric flasks.

c) Chromatographic development and scanning

Appropriate aliquots of standard and test solutions were applied to coated silica gel 60 F_{254} aluminium plates (10 * 10 cm, 100 µm thickness) using a Camag Linomat V sample applicator, forming 6 mm bands at a distance of 10 mm from the lower edge and 15 mm from the lateral margins. The mobile phase comprised toluene, methanol, ethyl acetate, and ammonia in a volume ratio of 5.5:2.3:2.2:0.05 (% v/v/v/v), with a chromatographic run length of 8.5 cm. The solvents were blended before use, and the development chamber was presaturated with mobile phase vapors for 20 min. prior to plate development. The ascending development technique was employed until a migration distance of 85 mm was reached. Following development, the TLC plates were airdried. Densitometric scanning was conducted in reflectance absorbance mode at 231 nm, with a slit dimension of 6.0 × 0.30 mm, a scanning speed of 20 mm/s, and a resolution of 100 µm per step. A deuterium lamp, providing a continuous UV spectrum from

200 to 400 nm, served as the radiation source. Drug concentrations were quantified based on the intensity of diffusely reflected light, and peak areas were analysed using linear regression.

D) method validation

Method validation conducted with reference to ICH Q2 (R1) recommendations, evaluating key performance characteristics such as linearity, accuracy, precision, robustness, specificity, as well as the limits of detection and quantification [50]. Linearity was assessed by analysing six replicates at each concentration within the limits of 2000–12,000ng/band for TNG and 1500–9000 ng/band for PIO, evaluating the correlation between peak area and drug concentration. To establish linearity, calibration curves were prepared and analysed using standard regression techniques. Method precision was determined by repeatability tests and intermediate precision assessments.

Repeatability was evaluated on the same day, and intermediate precision was assessed on different days by analysing three replicates of three concentrations: 4000, 6000, and 8000 ng/band for TNG, and 3000, 4500, and 6000 ng/band for PIO. Analyses were conducted in triplicate, and the %RSD of the peak areas was determined. Accuracy was assessed through recovery experiments at 80%, 100%, and 120% levels, by spiking the TNG (4000ng/band) and PIO (3000ng/band) dosage forms with three different amounts of the respective standards Solution (TNG: 3200, 4000, 4800 ng; PIO: 2400, 3000, 3600 ng) using the conventional standard addition procedure.

Retrieval studies were conducted in triplicate. In association with ICH guidelines, the LOD and LOQ were calculated using the standard deviation of the response and the slope of the calibration curve, applying the formulas LOD = 3.3 × (s/S) and LOQ = 10 × (s/S), where *s* denotes the standard deviation of the response and *S* the slope. Specificity was assessed by evaluating peak purity for both reference standards and the tablet formulation. Identification of TNG and PIO in the sample was confirmed by comparison of their Rf values and spectral profiles with those of the respective standards. Peak purity analysis for TNG and PIO was performed by comparing spectra at three levels of the chromatographic peak: start (S), apex (M), and end (E). The robustness of the method was defined as its ability to remain unaffected by minor, intentional variations in analytical conditions. Robustness was investigated using a fourfactor, half fractional (2^{4-1}) fractional factorial design (FFD). Factors were chosen based on trial runs, chromatographic intuition, and insights from previous studies, including ethyl acetate content in the mobile phase (A), solvent front position (B), detection wavelength (C), and chamber saturation time (D). To evaluate the impact on the response, Rf, the studied factors were purposefully modified from the optimum chromatographic conditions, enabling quantitative analysis of deviations for both medications. [Table 1] The four factors and their deliberate high- and low-level variations are presented. All experiments were conducted in a randomized order to minimize bias from un controlledvariables. Responses, recorded as the retention factors (Rf) of TNG and PIO, were used to evaluate the robustness of the method.

Table: 1 Experimental factor and assigned levels used for the FFD study.

Factors	High Level	Low Level
Ethyl Acetate	2.4	2
Chamber saturation time	18	22
Wavelength	233	229
Solvent front	82	78

e) analysis of marketed formulation

The content of twenty tablets (TNG 20 mg and PIO 15 mg) was weighed and finely powdered. A quantity equivalent to 20 mg of TNG was transferred to a 25 mL volumetric flask, dissolved in methanol, and sonicated for 30 minutes before making up the volume with methanol. The resulting solution was filtered through 0.45 µm Whatman filter paper to prepare a stock solution of 800 µg/mL TNG and 600 µg/mL PIO. Sample solutions of TNG (4000, 6000, 8000 ng/band) and PIO (3000, 4500, 6000 ng/band) were applied to HPTLC plates, followed by development and densitometric scanning at 231 nm. All measurements were carried out in triplicate.

f) Statistical analysis

The experimental design data were processed through DesignExpert version 7.0.0 (StatEase Inc., Minneapolis, USA), and further statistical analyses were carried out in Microsoft Excel 2013.

Result and discussion

The analysis of both drugs was carried out at a detection wavelength of 231 nm. Mobile phase optimization was conducted by testing various solvent systems and ratios, including nhexane, toluene, methanol, ethyl acetate, acetonitrile, diethyl ether, chloroform, and dichloromethane, to identify the most suitable conditions for separation. Mixtures of toluene, methanol, and ethyl acetate yielded effective separation, prompting additional trials with varied solvent ratios and the addition of modifiers including glacial acetic acid, ammonia, formic acid, triethylamine, and dimethylamine to optimize chromatographic performance. The addition of ammonia to the mobile phase improved chromatographic band shape and clarity. The final mobile phase, comprising toluene, methanol, ethyl acetate, and ammonia in the ratio 5.5:2.3:2.2:0.05 (v/v/v/v), produced strong, symmetrical peaks for both analytes.

TNG and PIO produced wellresolved bands at Rf values of 0.561 (2000 ng/band) and 0.789 (1500 ng/band), respectively, under conditions of 20minute chamber saturation with the mobile phase at room temperature and scanning at 231 nm. [Figure 4]. Within the studied concentration ranges (TNG: 2000–12,000 ng/band; PIO: 1500–9000 ng/band), both drugs showed excellent linearity (Table 2). The LODs for TNG and PIO were 233.20 µg/mL and 234.74 µg/mL,

respectively, while the LOQs were 706.69 ng/band and 711.34 ng/band, indicating the method's high sensitivity. Method precision was determined by evaluating repeatability and intermediate precision, expressed as %RSD of the peak area. The method showed good precision, with three replicates of three concentration levels (TNG: 4000, 6000, 8000 ng/band; PIO: 3000, 4500, 6000 ng/band) yielding %RSD values below 2% (Table 2). Accuracy, evaluated through standard addition, resulted in recoveries ranging from 99.13% to 100.90%, indicating the method is reliable and applicable for routine analysis of both drugs (Table 2). Peak purity for TNG and PIO in marketed tablet formulations was assessed by comparing the spectra at the start, apex, and end of the peaks with those of the standards [Figures 5A–5B], demonstrating excellent correlation. Correlation coefficients for TNG were r (S, M) = 0.99950 and r (M, E) = 0.99299, while for PIO, r (S, M) = 0.99995 and r (M, E) = 0.99978. Robustness studies were conducted in randomized sequence to reduce potential bias from uncontrolled factors. Graphical analyses, including response surface and perturbation plots, revealed the influence of experimental factors on the retention factors of TNG and PIO. Factor significance was evaluated using a Pareto chart: effects beyond the Bonferroni limit were deemed highly significant, effects above the tvalue limit were potentially significant, and those below the tvalue limit were considered insignificant.

The Pareto chart highlights that the solvent front has the most pronounced effect on PIO retention time and must be tightly controlled. [Figures 6A–6B] indicate the decreasing order of factor effects on Rf: for TNG, B > D > A > C > A; for PIO, B > C > A > D. Perturbation plots display deviations in response from the nominal value while keeping other parameters fixed, with steep slopes or curvatures identifying high sensitivity to specific factors. [Figures 7A–7B] illustrate that small changes in solvent front and chamber saturation time significantly impacted PIO but had minimal effect on TNG. Three-dimensional response surface plots confirm that TNG Rf remained largely unaffected by the factors, while PIO Rf varied with saturation time (Figures 8A–8F). The experimental model was validated through ANOVA using Design Expert software (Tables 3 and 4). Responses at specific factor levels can be predicted using the coded factor equation, which also facilitates evaluation of the relative importance of each factor through coefficient comparison. A value above 0.05 indicates that the covariates did not significantly influence the response, confirming method robustness. Both TNG and PIO exhibited adequate precision — the signal to noise ratio exceeded 4 — and the low coefficient of variation (%CV) along with satisfactory accuracy indicate that the observed results closely match those predicted by the model.

The Zita Plus Pio tablet formulation, containing 20 mg Teneligliptin and 15 mg Pioglitazone, exhibited good recovery when analysed in triplicate using the proposed HPTLC method. The drug content ranged from 98.83 to 100.32% with %SD below 2 (Table 5), confirming the method's suitability for routine quality control. The developed method proved to be straightforward, accurate, precise, specific, and reproducible for simultaneously determining teneligliptin (TNG) and pioglitazone (PIO) in tablet form. Compared to HPLC, the HPTLC approach allows multiple samples to be analyzed in parallel using less mobile phase, which reduces both analysis time and persample cost.. Robustness was examined using fractional factorial design to study the simultaneous variation of factors and their effects on responses.

Among the variables, the solvent front in the mobile phase significantly affected TNG response but had nonsignificant effects on PIO, emphasizing the importance of precise control. Employing experimental design along with response surface methodology provides a flexible framework for reducing the number of experiments needed to evaluate robustness. The developed method was repeatable and appropriate for routine analysis of combined dosage forms.

Table 2: Analytical Validation Parameters for Simultaneous Determination of TNG and PIO Using the Proposed HPTLC Method

Parameters	**TNG**	**PIO**
Linear regression parameters		
Calibration range[a] (ng/band)	200012000	150009000
Regression equation	y = 0.000001x + 0.002	y = 0.0000002x + 0.0061
Regression coefficient	0.9963	0.9967
Standard deviation of slope	0.00000001170	0.00000002494
Confidence limit of slope[b]	0.00000130 0.00000134	0.00000175 0.00000179
Standard deviation of intercept	0.00009357	0.00012661
Confidence limit of intercept[b]	0.00190.0021	0.00590.0062
Sensitivity		
Limit of detection (µg/ml)	233.20	234.74
Limit of quantification (µg/ml)	706.69	711.34
Precision		
Repeatability (%RSD) [c]	0.72	1.01
Intraday precision (%RSD) [d]	0.550.79	0.460.62
Interday precision (%RSD) [d]	1.041.90	1.011.25
Accuracy		
80 % (Mean% recovery ± %RSD) [d]	99.37 ± 0.90	100.12 ± 0.95
100 % (Mean% recovery ± %RSD) [d]	99.94 ± 1.77	99.76 ± 0.76

120 % (Mean% recovery ± %RSD) [d]	100.01 ± 1.03	99.13 ± 1.44
Specificity		
r (S, M)	0.999874	0.999950
r (S, M)	0.990697	0.999514

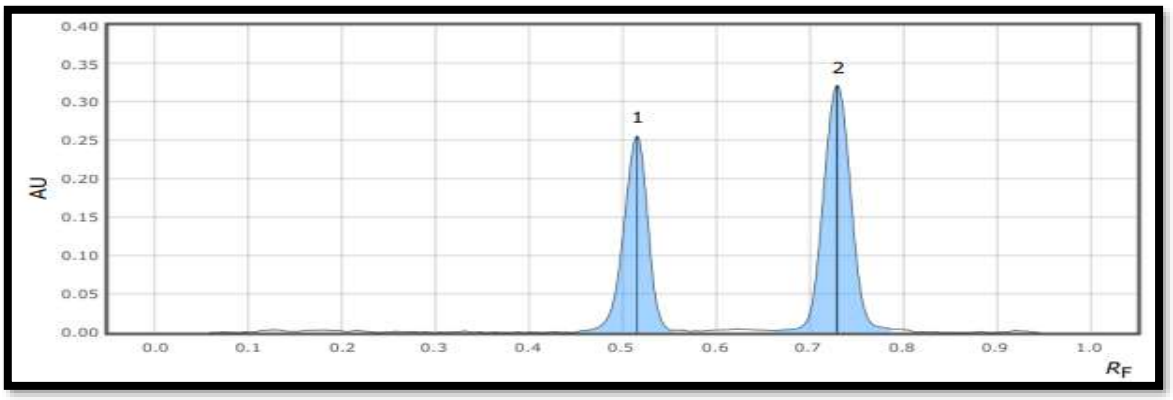


Figure. 4 – TLC Chromatogram of standard: TNG (Rf 0.561) and PIO (Rf 0.789). TNG – Teneligliptin; PIO – Pioglitazone = thin layer chromatography.

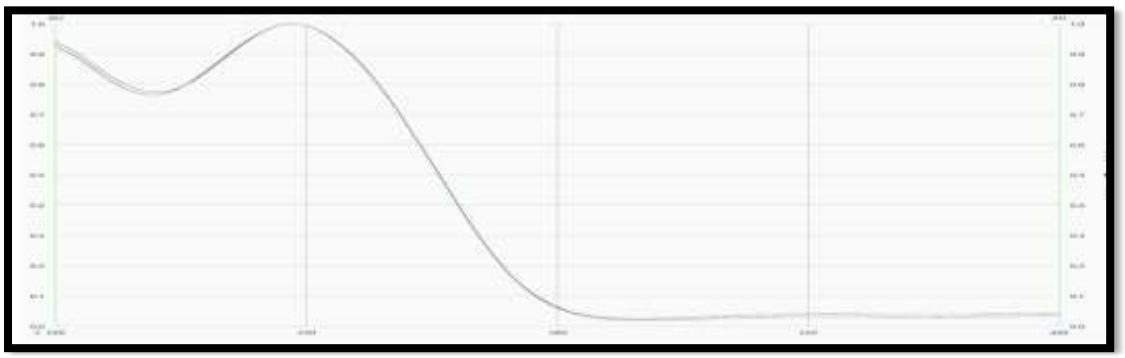


Figure 5A

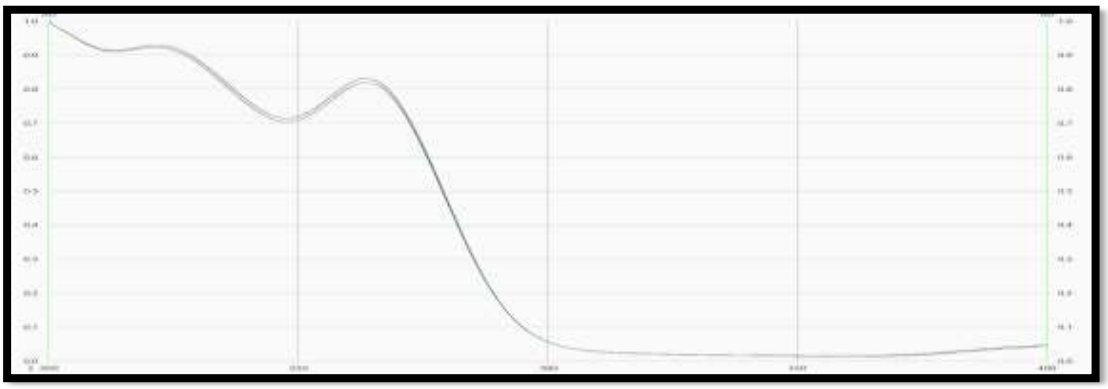


Figure 5B

Figure 5: Overlaid spectra of samples with standard showing peak purity, TNG (5A) and PIO (5B)

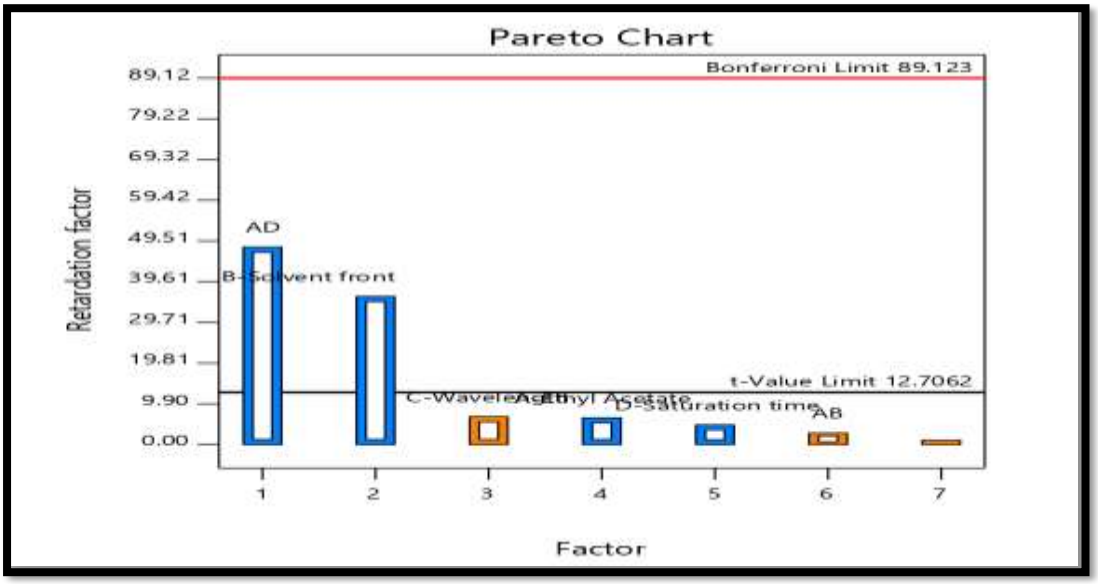


Figure 6A

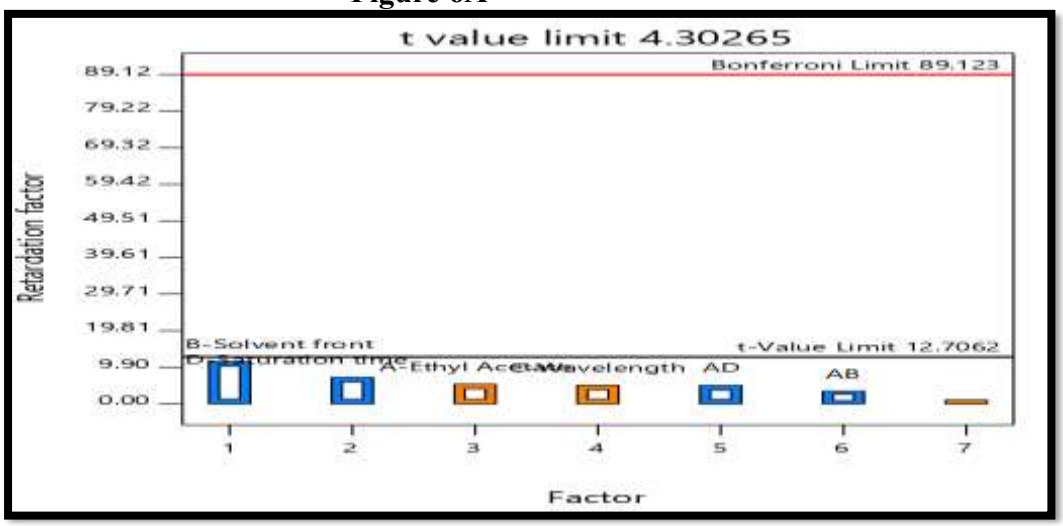
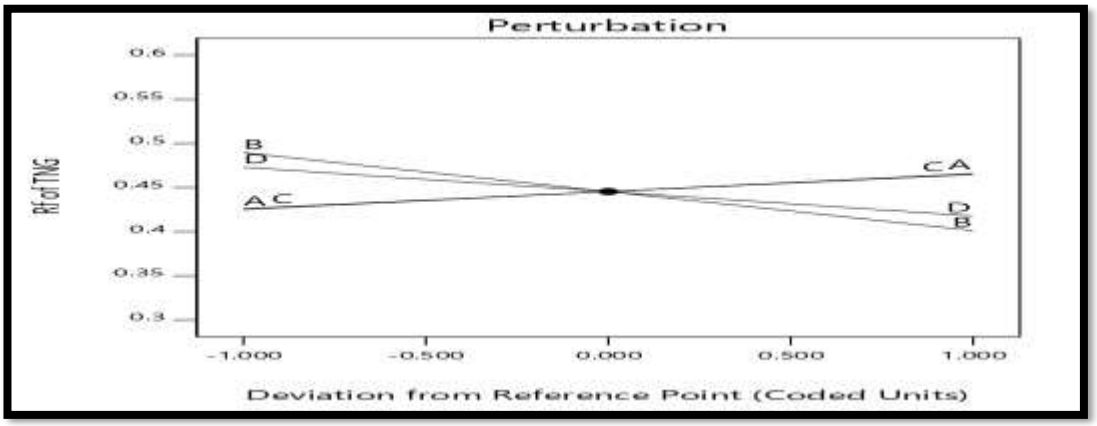


Figure 6B

Figure 6: Pareto chart showing the effect of factors and interaction on Rf values of TNG (6A) and PIO (6B)



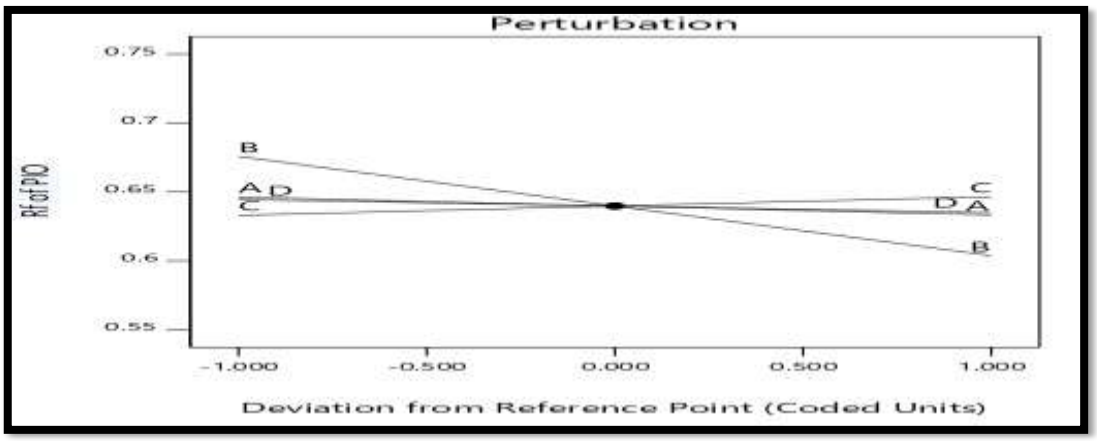


Figure 7: Perturbation plot showing effect of factors on Rf values of TNG (7A) and PIO (7B)

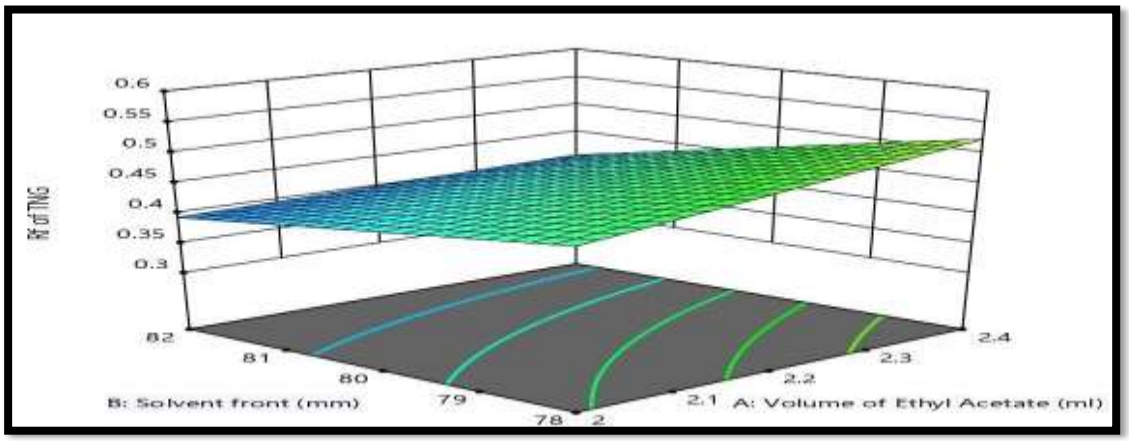


Figure 8(A)

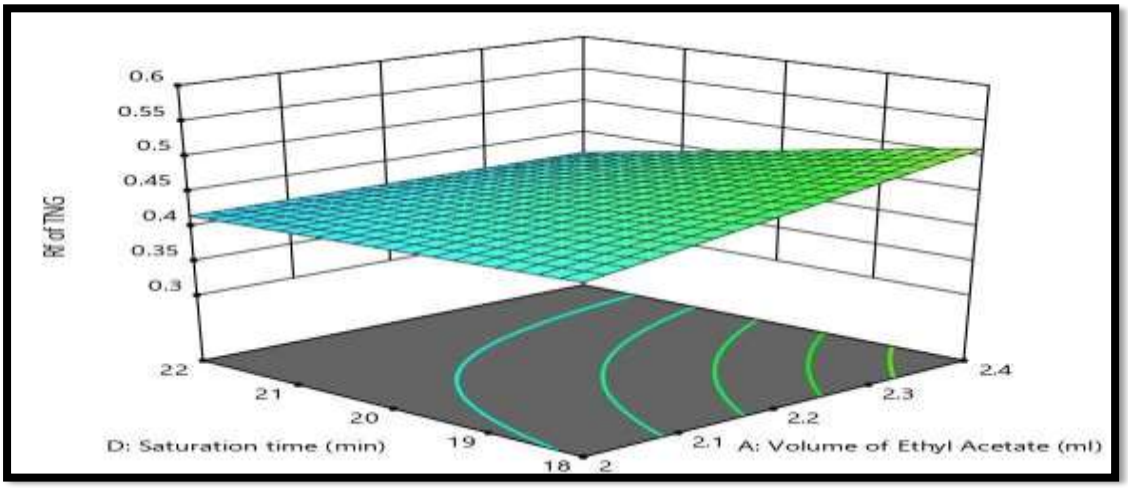
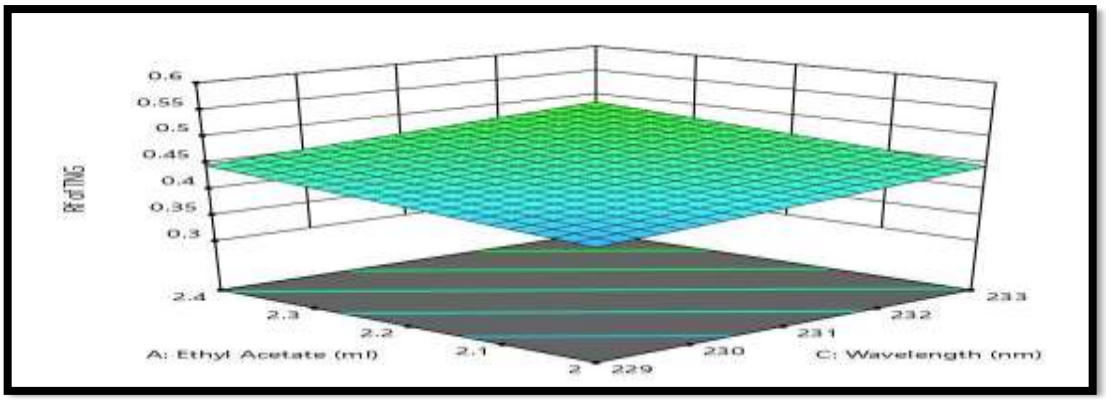


Figure 8(B)



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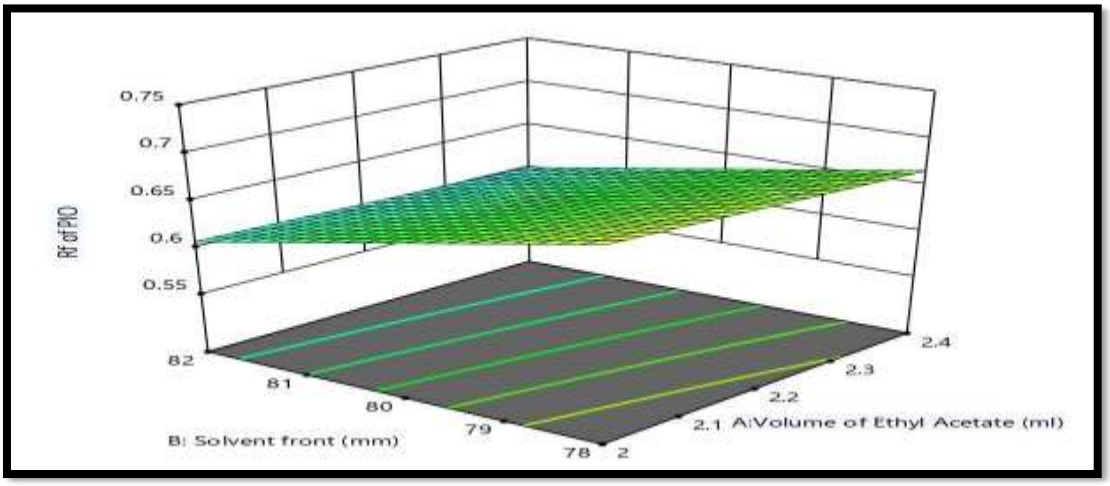

Figure 8(D)

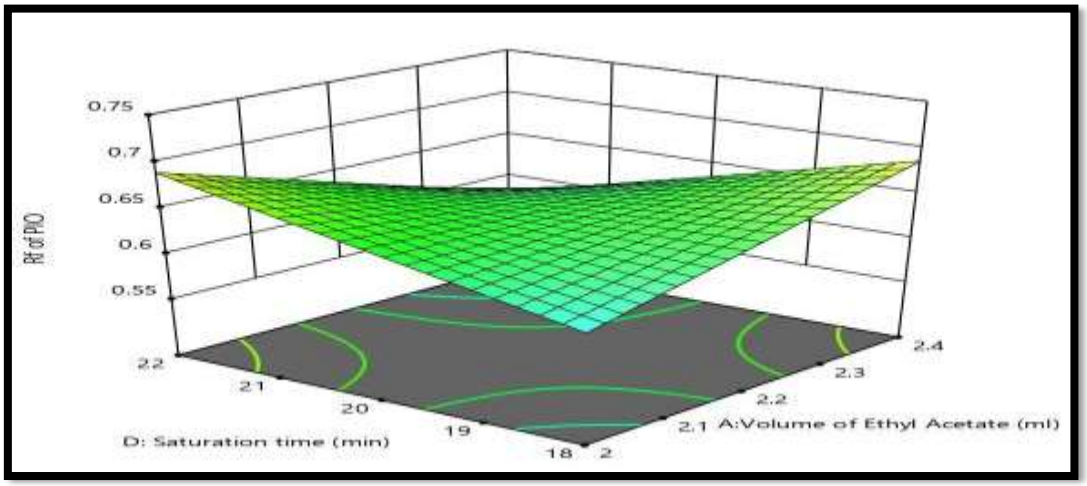

Figure 8(E)

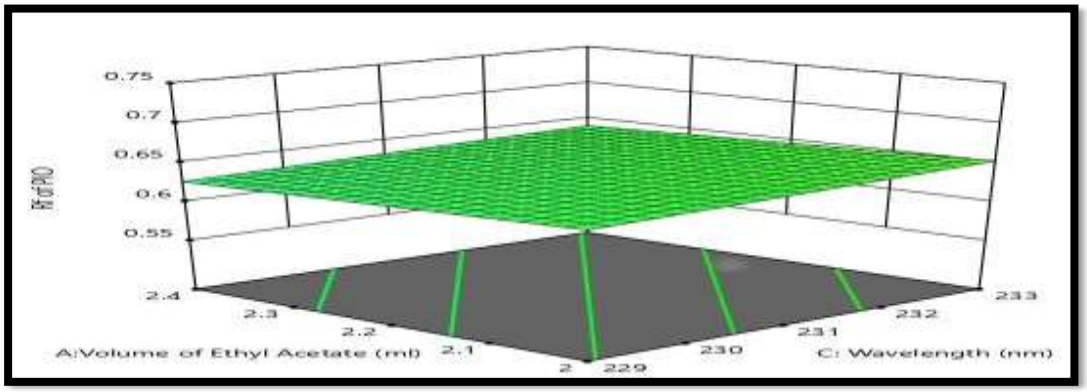

Figure 8(F)

Figure 8: Three-dimensional response surface plot showing effect of factors on Rf values of TNG AND PIO

Table 3 – Predicted response model and statical parameters by ANOVA analysis

Responses	**Std. Dev.**	**Mean**	**C.V %**	**RESS**	**R Squared**	**Adj R Squared**	**Predicted R Squared**	**Adequate Precision**
R*f* of TNG	0.0110	0.445	2.46	0.0077	0.9963	0.974	0.7269	21.75
R*f* of PIO	0.0028	0.639	0.44	0.0005	0.9997	0.998	0.9828	68.41

Table 4 – Polynomial equation for responses

Sr. No.	**Response**	**Polynomial equation**
1	R*f* of TNG	0.4454 + 0.0204A – 0.0441B + 0.0194C – 0.0276D 0.0131AB 0.0191AD
2	R*f* of PIO	0.6398 0.0065A 0.0360B + 0.0067C 0.0048D +0.0028AB0.0480AD

Table 5: Analysis of Marketed formulation

Drug	Label Claim (mg)	Conc. (ng/band)	% Amount found			Mean % Amount found	S.D.	% RSD
			1	2	3			
TNG	20 mg	4000	101.15	99.64	98.69	98.83	1.23	1.24
		6000	100.66	101.29	99.02	100.32	1.16	1.16
		8000	98.91	98.81	98.43	98.72	0.24	0.24
PIO	15 mg	3000	99.11	100.80	98.18	99.36	1.32	1.33
		4500	100.04	101.04	99.67	100.25	0.71	0.70

CONFLICT OF INTEREST

The authors have no conflict of interest regarding this investigation

ACKNOWLEDGMENTS

The authors gratefully acknowledge Precise Chemipharma Pvt. Ltd., Vadodara, Gujarat, and Abhilasha Pharma, Ankleshwar, India, for kindly providing standard samples of teneligliptin and pioglitazone, respectively.

CONCLUSION

In this study, a high-performance thin layer chromatography (HPTLC) method was developed and validated for the simultaneous estimation of teneligliptin (TNG) and pioglitazone (PIO) in tablet formulations, adhering to ICH Q2(R1) guidelines. Silica gel 60F254 was utilized as the stationary phase, and the mobile phase consisted of toluene, methanol, ethyl acetate, and ammonia in a 5.5:2.3:2.2:0.05 (v/v/v/v) ratio. This mobile phase composition was optimized and employed consistently throughout the method validation and analysis of the marketed formulation. A fractional factorial design (2^{4-1}) was applied to assess the robustness of the method. The retention factor (Rf) of TNG remained unaffected by variations in factors such as ethyl acetate volume, solvent front, wavelength, and chamber saturation time, indicating method robustness. However, for PIO, the solvent front demonstrated a significant impact on robustness, highlighting the necessity for careful control of this parameter.

References

[1] Deshmukh CD, Jain A, Nahata B. Diabetes mellitus: a review. Int. J. Pure Appl. Biosci. 2015;3(3):22430.
[2] Singh N, Kesherwani R, Tiwari AK, Patel DK. A review on diabetes mellitus. The Pharma Innovation. 2016 Jul 1;5(7, Part A):36.
[3] Pradeepa R, Mohan V. Epidemiology of type 2 diabetes in India. Ind. J. Ophthal. 2021 Nov;69(11):2932.
[4] Sathananthan A, Vella A. Personalized pharmacotherapy for type 2 diabetes mellitus. Personalized Medicine. 2009 Jul;6(4):41722.
[5] International Conference on Harmonization, ICH Q2 (R1): validation of analytical procedures; text and methodology Geneva. 2005, 117.
[6] Shah R, Pawar R, Gayakar P, UVVisible spectroscopy A review Int. Inst. Pharm. Life Sci. 2015, 5(5), 490505.
[7] Kamal AH, Malla SF, Hammad SF, "A review on UV spectrophotometric method forsimultaneous multicomponent analysis." Eur. J. Pharm. Med. Res. 2016, 3(2), 348360.
[8] Modi V, Bhatkar S, Prajapati P, Basuri T, "A review on various application of high-performance thin layer chromatography (hptlc) in pharmaceuticals." Int. J. Innov. Pharm. Sci. Res. 2016, 4(3), 384400.
[9] Kumar V, Kamle P, Mithal A, "High performance thin layer chromatography (hptlc): a review." Int. J. Anal. bioana. Chem. 2014, 4(2), 4244.
[10] Srivastava MM, "High performance thinlayer chromatography (HPTLC), Published by springer." 2011, 11, 4649.
[11] Kumar V, Kamle P, Mithal A, "High Performance Thin Layer Chromatography (HPTLC): A Review." Int. J. Anal. Bioana. Chem. 2014, 4(2), 4244.
2[12] Yadav N, Goyal A. Method development and validation of Teneligliptin in pharmaceutical dosage form by UV Spectrophotometric methods. Int J Pharm Chem and Anal. 2017; 4:548.
[13] Jain P, Patil P, Sonawane S & Surana S. UVAUC spectrophotometric method for quantitative estimation of teneligliptin. Acta scientific pharmaceutical sciences; 2019;3(6):4347.
[14] Kshirsagar S, Mane S, Hanchate Y, Katte A, Kulkarni K. UV Spectrophotometric Method Development and Validation for Determination of Teneligliptin Hydrobromide Hydrate in API and in Pharmaceutical Dosage Form. Int. J. Pharm Res. Schol.2018;7(1):1927.
[15] Hemke A, Rathod E, Gupta K, Umekar M. HPLC And UV Spectrophotometric Estimation of Teneligliptin from Tablet Dosage Form. Asian J. Pharm. Anal. and Med. Chem. 2016;4(3):148156.
[16] Lokhande P. Analytical method development and validation of teneligliptin by using RPHPLC with ICH guidelines. Int. J. Sci. Res. Develop. 2019 March April;3(3):259263.
[17] Chandana M, Rao MP, Samrajyam B, Sireesha KS, Premi VV. Analytical method development and validation of teneligliptin in pharmaceutical dosage form

by RPHPLC method. J Health Sci Nurs. 2016; 1:12.
[18] Kumar TN, Vidyadhara S, Narkhede NA, Silpa YS, Lakshmi MR. Method development, validation, and stability studies of teneligliptin by RPHPLC and identification of degradation products by UPLC tandem mass spectroscopy. J. Anal Sci. Technol. 2016 Dec;7(1):12.
[19] Luhar SV, Pandya KR, Jani GK, Sachin B, Narkhed S. Simultaneous estimation of teneligliptin hydrobromide hydrate and its degradation product by RPHPLC method. J Pharm Sci. Biosci. Res. 2016;6(3):25461.
[20] Patil PM, Jain PS, Surana SJ, Yeole SD. Development and validation of High performance thinlayer chromatography method for estimation of Teneligliptin in bulk and In Pharmaceutical Formulation. Acta chemical and pharmaceutical indica. 2017;7(3):18.
[21] Lodha SR, Patel KD, Patel SA, Patel SG, Bodiwala KB, Shah SA, Kalyankar GG. Development and validation of HPTLC method for estimation of teneligliptin hydrobromide hydrate in tablet dosage form. J. Pharma. Appli. Sci.June. 2016;3(1):27.
[22] Shinde V, Aher K, Bhavar G, Kakad S, Chaudhari S. Development and validation of UV spectrophotometric method and highperformance thin layer chromatographic (HPTLC) method for estimation of teneligliptin hydrobromide in pharmaceutical preparation. Scholars Research Library.2016; 8(8):291301.
[23] Bisht P, Gahtori A. Development and Validation Of UVVisible Spectrophotometric Method for Simultaneous Determination of Teneligliptin Hydrobromide and Metformin Hydrochloride in Its Bulk and Dosage Form. J. Emerge. Technol. Innov. Res. 2020 June;7(6):988995.
[24] Sen AK, Hinsu D, Sen D, Zanwar A, Maheshwari R, Chandrakar V. Analytical method development and validation for simultaneous estimation of Teneligliptin hydrobromide hydrate and Metformin hydrochloride from it's pharmaceutical dosage form by three different UV spectrophotometric methods. J. Appli. Pharm. Sci.2016 Sept;6(9):157165.
[25] Vyas A, Godhaniya J, Patel A, Patel A, Patel N, Shah S, Sheth D. Development and Validation of UVSpectroscopic First Order Derivative Method for Simultaneous Estimation of Rosuvastatin Calcium and Teneligliptin Hydrobromide Hydrate in Synthetic Mixture. Chemical Methodologies. 2020;5(4):31723.
[26] Attimarad M, Venugopala KN, Nair AB, Sreeharsha N, Deb PK. Experimental design approach for quantitative expressions of simultaneous quantification of two binary formulations containing remogliflozin and gliptins by RPHPLC. Separations. Jan 2022;19;9(2):23.
[27] Irache GS, Bhajipale NS, Gandhi LR. RPHPLC method development and validation of teneligliptin and metformin in pharmaceutical dosage forms. Int Res J Pharm. 2017;(8):525.
[28] Patel F, Dalwadi P, Patel P, Mehta N. Development and validation of RPHPLC method for simultaneous estimation of teneligliptin hydrobromide hydrate and rosuvastatin calcium in synthetic mixture. Int. J. Res. Edu. Sci. Method. 2021 April;9(4):27122718.

[29] Patel M, Patel D, Shah U, Kachhiya H. Simultaneous quantification of teneligliptin hydrobromide and metformin hydrochloride: an improved HPTLC method with implementation of PlackettBurman design. J. Chem. Metrology. 2021 Jan 1;15(1).
[30] Chandra A, Rathod R, Ali F, Prakash A, Kumar R, Singh GN. Development and validation of a rapid and sensitive method for the simultaneous estimation of gemigliptin and teneligliptin in bulk and dosage forms by using liquid chromatographytandem Mass spectrometry. Current Pharmaceutical Analysis. 2020 Dec 1;16(8):110411.
[31] The Indian Pharmacopoeia, Government of India, Ministry of Health and Family Welfare the Indian Pharmacopoeia commission, Ghaziabad, 2018; 3: 2933.
[41] The United States Pharmacopoeia. The National Formulary. Rockville, Md.: United States Pharmacopeial Convention, Inc., 2015: 48864887.
[32] Shakya P, Singh K. Determination of pioglitazone hydrochloride in bulk and pharmaceutical formulations by UV spectrophotometric method. Int. J. of Pharmaceutica. Sci. Res. 2010 Nov 1;1(11):153.
[33] Naveed S, Ashraf Z, Mukhtar T. Assay of pioglitazone HCL by using UV visible spectrophotometer. Br. J. Res. 2015;2(1).
[34] Hema VV, Tiwari BR. Method development of pioglitazone by UV spectrophotometer. Int. J. Drug Dev. Res. 2014;6(4):8083.
[35] Chandana R, Bhavya sri K, Sumakanth M, Swethasri R. Analytical method development and Validation for the estimation of pioglitazone hydrochloride by using RPHPLC. Int. J. Sci. Technol. Res. 2019 oct;8(10).
[36] Meeta A Jiladia, SS Pandya, Viidyasagar G. A simple and sensitive HPTLC method for estimation of Pioglitazone in bluk and tablet dosage forms. Asian J Res. chem. 2009;2(2):207209.
[37] Kottu PK, Gadad AP, Dandagi PM. Method development and validation for the simultaneous estimation of Pioglitazone and glimepiridea UV spectrophotometric approach. Indian drugs. 2012 Nov;49(11):1728.
[38] Sen DB, Sen AK, Zanwar AS, Pandey H, Maheshwari RA. UV Spectrophotometric methods to quantify alogliptin benzoate and pioglitazone hydrochloride. Int. J. Pharm. Res. July 2021:33(37B):3141.
[39] Sujana K, Swathi Rani G, Bhanu Prasad M, Reddy MS. Simultaneous estimation of pioglitazone hydrochloride and metformin hydrochloride using UV spectroscopic method. J Biomed Sci Res. 2010;2(2):1105.
[40] Joshi RS, Nangare AK, Sanap DS, Sase SM. Development and validation of UVspectrophotometric method for estimation of metformin hydrochloride and pioglitazone in tablet dosage form. J. Drug Deliv. Ther. 2019 Aug 30;9(4A):3814.
[41] Dhani R, Guptha HK, Rajamanickam D. Development of stability indicating method for the simultaneous estimation of alogliptin and pioglitazone in bulk and tablet dosage form by reversedphase ultraperformance liquid chromatography method. J. Appli. Pharm. Sci. 2019 Dec 3;9(12):0516.
[42] Elrefay H, Ismaiel O, Hassan W, Shalaby A. Development and validation of a stability indicating HPLCUV method for the determination of pioglitazone

hydrochloride and metformin hydrochloride in bulk drug and Combined dosage form. Asian J. Pharma. Clin. Res. 2013 July 21;6(4).
[43] Kumar VP, Sunandamma Y. Simultaneous determination of clopidogrel and pioglitazone by high performance liquid chromatography in bulk drug and dosage forms. Int. J. Pharm. Life Sci. 2013 Apr 15;2(1):19.
[44] Gajul V, Tegeli Thakkar N, Patil S, Kalshetti M. Development and validation of analytical methods for simultaneous estimation of sitagliptin phosphate and pioglitazone hydrochloride. Int. J. Pharm. Res. 2018;7(2):1222.
[45] Anand DP, Senthilkumar KL, Senthilkumar B, Saravanakumar M, Thirumurthy R. HPTLC method for determination of telmisartan HCl with pioglitazone HCl in pharmaceutical dosage form. Int. J. Pharm. Res. 2010; 2:18590.
[46] Khorshid A, Abdelhamid NS, Abdelaleem EA, Amin MM. Simultaneous determination of metformin and pioglitazone in presence of metformin impurity by different spectrophotometric and TLC–densitometric methods. SOJ. Pharm. Sci. 2018;5(3):18.
[47] Varade R, Mishra H. A rapid validated unidimensional double development HptlcDensitometrymethod for simultaneous Estimation of metformin hydrochloride, gliclazide and pioglitazone hydrochloride. Int J Pharm Sci. 2019.
[48] Sondarva C, Shah K. Development and Validation of HPTLC method forsimultaneous estimation of antidiabetic drugs from their combined dosage form. Int.J. Pharm. Res. 2018 Jan; 11(2):193210.
[49] Gheewala A, Maheshwari D. Development and Validation of UV Spectrophotometric Method and RPHPLC Method for Simultaneous Estimation of Teneligliptin and Pioglitazone in Synthetic Mixture. 2017;5(23):6678.
[50] Guideline ICH. Validation of analytical procedures: text and methodology. Q2 (R1): validation of analytical procedures: text and methodology 2005