Current Trends in Biotechnology and Pharmacy
Vol. 19 (Supplementry Issue 1A), June 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2025.2s.2

A Quality by Design Driven RP-HPLC Method for the Assay of Lobeglitazone & Glimepiride and *In-silico* Admet Studies

Gangu Naidu Challa[1], Jagadeesh Adari[2], Vara Prasada Rao Kollabathula[3], Srinivasa Rao Yarguntla[3], and Bhagavan Rajesh Babu Koppisetty[3]*

[1]Vignan's Institute of Information Technology, Visakhapatnam - 530 046, Andhra Pradesh, India
[2]GITAM University, Visakhapatnam - 530 045, Andhra Pradesh, India
[3]Vignan Institute of Pharmaceutical Technology, Visakhapatnam – 530 046, Andhra Pradesh, India
*Corresponding author: koppisettybrbabu@gmail.com

Abstract

A cost-effective, simple and accurate Quality by Design (QbD) based approach for the analysis of Lobeglitazone and Glimepiride in tablet dosage forms was developed. In-silico study was performed to investigate the pharmacokinetic and toxicological properties of the two drug products.The method was subjected to optimization using a central composite design, where column temperature, flow rate, and organic phase ratio in the mobile phase were optimally tuned as critical parameters to determine critical analytical parameters, i.e., resolution and theoretical plate number. Maximum separation was achieved on a C18 column using a mobile phase containing acetonitrile and 10 mM ammonium acetate buffer, 1.0 mL/min flow rate, and 40 °C column temperature. The statistical assessment was done using Design expert software and excel. pkCSM web server was used for Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) studies. Glimepiride and Lobeglitazone retention times of 8.923 minutes and 10.529 minutes, respectively, were achieved. The drug product was found to be sensitive towards acidic, basic, neutral, oxidative, photolytic, and thermolytic conditions. Glimepiride's potential for hepatotoxicity and Lobeglitazone's possible cardiac risks due to hERG inhibition highlight the need for vigilant monitoring in patients.

Keywords: Lobeglitazone, Glimepiride, Quality by design, RP-HPLC, *in-silico*

Introduction

Glimepiride (GPR) and lobeglitazone (LGL) are used to regulate blood sugar levels in people with type 2 diabetes mellitus (T2DM). GPR, a sulfonylurea, is taken as tablets and is a white to yellowish-white crystalline powder with no discernible smell. It works by encouraging the release of insulin from beta cells in the pancreas.GPR binds to specific receptors on these cells, leading to the closure of potassium channels, which causes cell depolarization and a subsequent influx of calcium ions. This influx triggers insulin release, thus lowering blood glucose levels. GPR is a highly potent and long-acting third-generation sulfonylurea, more efficient and longer-lasting than other drugs in its class. It is metabolized by CYP2C9 and also acts as an agonist for peroxisome proliferator-activated receptor gamma (PPARγ). Chemically, GPR is described as "1-[[4-[2-(3-Ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)-ethyl]phenyl]sulfonyl]-3-trans-(4-methylcyclohexyl)urea" (1,2).LGL (Figure 1) is a newer addition to the thiazolidinediones (TZDs) class, which is primarily used to improve glycemic control in T2DM patients. As a PPARγ agonist, LGL enhances insulin sensitivity in adipose tissue, muscle, and the liver. It has drawn attention due to its potentially favorable safety and efficacy profile, providing effective glycemic control with fewer side effects, particularly cardiovascular risks, compared to other TZDs like rosiglitazone and pioglitazone. Chemically, LGL is described as "5-[[4-[2-[[6-

Vol. 19 (Supplementry Issue 1A), June 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2025.2s.2

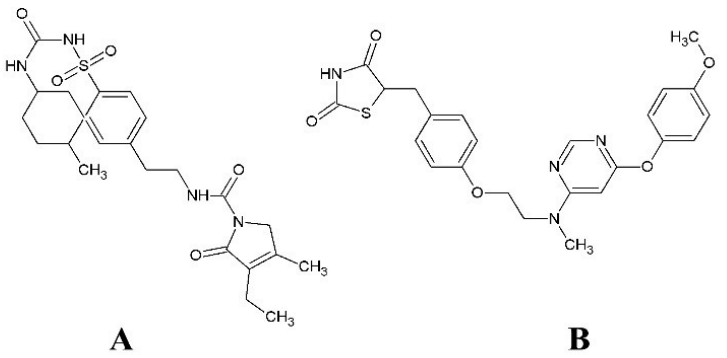

Figure 1: Chemical structures of Glimepiride (A) and Lobeglitazone (B)

(4-methoxyphenoxy)pyrimidin-4-yl]-methylamino]ethoxy] phenyl]methyl]-1,3-thiazolidine-2,4-dione" (3,4,5).GPR has been individually analyzed using UV spectrophotometry (6), HPLC (7,8,9,10), and LC-MS (11) in both plasma and pharmaceutical formulations. Furthermore, LC/MS/MS (12) has been utilized for its quantification in biological matrices, while LC-MS (13) has been employed to characterize its degradation products. Similarly, LGL has been independently assessed through HPLC (14) in formulations and plasma and quantified in biological matrices using LC/MS/MS (15). Additionally, LC-MS (16) has been used to study the degradation products of LGL. Analytical methods such as HPLC (17), HPTLC (18), and UV spectroscopy (19) have been reported for the analysis of GPR and LGL in drug formulations. However, no documented research exists on a stability-indicating, QbD-based RP-HPLC method for the simultaneous determination of GPR and LGL in marketed formulations. Additionally we have conducted, computational ADMET studies in understanding their bioavailability, metabolic stability, and toxicity risks. This study employs pkCSM, a machine-learning-based tool, to compare the pharmacokinetic and toxicity profiles of Glimepiride and Lobeglitazone.

Materials and Methods

Reagents

GPR and LGL were generously provided as samples by Synpure labs India Pvt Ltd. The LOBG-G1 drug product (Glenmark) was sourced from the local market. Analytical-grade reagents, including ammonium acetate, NaOH, HCl, and H_2O_2, were obtained from Rankem Chemicals, India. Acetonitrile (ACN) HPLC grade was obtained from Merck, India.

Statistical assessment

The data were statistically analyzed using Excel 2007 to calculate mean, coefficient of variation (CV), linear regression and relative standard deviation (RSD). Design Expert software (version 13.0.5.0) was used to perform ANOVA, generate 3D surface response plots, and optimize models. pkCSM for ADMET studies.

Instrumentation

Chromatographic separation was performed using an HPLC system (LC-20AD) from Shimadzu, Japan, featuring a binary pump and a PDA detector. An injector equipped with a 20 µL sample loop was used, with data acquisition handled through LC Solution. Spectroscopic studies were conducted using a UV spectrometer from Lab

Vol. 19 (Supplementry Issue 1A), June 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2025.2s.2

India, India. An analytical balance (ML303T) from Mettler Toledo was employed for precise measurements.

Conditions for chromatography

Separation was done with an Enable C18 G column (5 µm particle size, 4.6 mm ID, 250 mm length). The mobile phase was a 65:35 v/v mixture of ACN and ammonium acetate buffer (AA_{BUFFER}) 10 mM at pH 3.5, with a rate of flow (F_R) at 1.0 mL/min and 40 °C column temperature of (C_T). A 15µL sample was injected, and eluents were detected at 235 nm.

Preparation of standard solutions

Both the pure drugs, GPR and LGL, were precisely weighed at 25 mg and placed in different 25 mL volumetric flask. To each of the flasks, 15 mL of ACN was added, and sonicatedthe mixture for 18 minutes. The volumes were then filled up to 25 mL using ACN to prepare the individual stock solutions. Working solution standard of 100 µg/mL for GPR and 50 µg/mL for LGL were set by serial dilution of the mobile phase. The 0.45 µm nylon filter was used for filtering the solutions.

Preparation of sample solution

To prepare a powder of 25 mg GPR and 12.5 mg LGL for the LOBG-G1 (GPR 1 mg/LGL 0.5 mg) analysis, fifty tablets were ground finely with a glass mortar, after which the powder obtained was transferred into a 25 mL of calibrated flask. Around 15 mL of ACN was added to the flask, and the mixture was sonicated for 18 minutes to completely dissolve it. The solution was diluted with ACN to get 50 µg/mL for LGL and 100 µg/mL for GPR. The concentration levels of GPR and LGL were then adjusted to 50% to 150% of the prescribed specification levels, and the solution was given an additional sonication time of 20 minutes. The solution obtained was filtered using a 0.45 µm nylon filter before being analyzed using a HPLC system. The quantity of GPR and LGL was calculated using the Central Composite Design (CCD) method.

Method development using Experimental design

The initial trials were performed through a experimentation to gain through insight of the method's efficiency and to determine the most important factors that affects the variable outcomes.ACN and water were used as solvent mobile phase to achieve efficient separation of LGL and GPR and system suitability (20,21,22). Chromatograms were captured under various flow rates and mobile phase compositions. Development of an HPLC method under AQbD involves identification of the critical factors and responses, based on initial trials and risk assessments (23,24). The chromatographic conditions were optimized using a CCD method (25,26). Based on a CCD model with three variable factors and three response factors, 17 different chromatographic conditions were generated, as presented in Table 1. To reduce uncontrolled variable effects and bias, the experiments were performed in randomized order. Through extensive analysis, the FR, ACN volume (ACNVol), and CT were recognized as the three most important factors. The ACNVol (Factor 1) was varied between 50 mL and 80 mL, whereas the CT (Factor 2) and FR (Factor 3) were kept at ranges of 25–55 °C and 0.5–1.5 mL/min, respectively. The acquired data were analyzed using Design Expert statistical software (Version 13.0.5.0, Stat-Ease Inc., USA).

Method Validation

In compliance with ICH guidelines and relevant studies (27,28,29) system suitability testing and validation parameters (30,31,32,34) were performed according to standardized protocols.

Forced degradation

Stress degradation studies (34,35,36) on the tablet formulation of GPR (LOBG-G1) and LGL were performed according to the International Council for Harmonisation (ICH) guidelines Q1A(R2). The tablet formulation was exposed to different stress conditions of acidity, alkalinity, oxidation, heat, and light with an

Vol. 19 (Supplementry Issue 1A). June 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2024.3s.1

aim to assess the suitability of the analytical method developed as a measure of stability. One hundred and twenty tablets were weighed accurately and then crushed into fine powder in order to facilitate easier degradation analysis. A rough equivalent of weight of 100 mg of GPR and 50 mg of LGL was added to a sterile and dry volumetric flask, which has a 100 mL capacity. Diluent to the volume of 70 mL was added for complete dissolution of the contents, followed by sonication of the solution for 30 minutes. The volume was then made correct by adding additional diluent. A 0.45-micron injection filter was applied to filter the stock solution. The drug was exposed to forced degradation by refluxing in 0.01 N HCl at 65°C, 0.01 N NaOH at room temperature, and distilled water at 75°C for 12, 72, and 30 hours, respectively. Oxidative stress testing was performed using 3% hydrogen peroxide at room temperature for 72 hours. Thermal stress testing was performed on the drug in solid and solution forms by exposing it to a temperature-controlled oven at 65°C for up to 72 hours. Photolytic stress testing was done by exposure of powder and liquid form of drug to the UV lamp for 72 hours.

In-silico ADMET analysis

The *in-silico* ADMET studies on LGL and GPR was performed to predict their pharmacokinetic and toxicity levels of these drugs using SMILES notation and pKCSM web server. The parameters tested for absorption included water solubility, $CaCO_2$ permeability, human intestinal absorption, skin permeability, and P-glycoprotein interactions. The distribution parameters included volume of distribution (VDss), blood-brain barrier (BBB) permeability, CNS permeability, and fraction of unbound drug. The metabolic nature of the drug was predicted by the action of the drug on CYP450 enzymes. The excretion parameters of the drug was predicted by using total clearance and renal elimination through

Table 1: Experimental runs and responses for CCD

Run	1F	2F	3F	1R	2R	3R
	A: ACN_{Vol} (mL)	B: C_T (°C)	C:Flow rate (mL/min)	GPR (N)	LGL (N)	R_S
1	65	55	1	12614	10371	2.57
2	65	40	1	9147	8152	3.79
3	50	25	1.5	3152	2469	6.91
4	50	55	1.5	3601	2867	5.83
5	80	55	0.5	3941	2828	2.94
6	80	55	1.5	13793	13181	0.14
7	80	25	1.5	12695	12539	0.33
8	65	40	1	9238	8194	3.76
9	65	25	1	7321	6684	3.87
10	50	40	1	1937	1618	12
11	80	40	1	13133	12676	0.12
12	65	40	1.5	10738	9692	2.68
13	50	25	0.5	994	837	17.22

1F: 1st Factor; 2F: 2nd Factor; 3F: 3rd Factor; 1R: 1st Response; 2R: 2nd Response; 3R: 3rd Response

Vol. 19 (Supplementry Issue 1A), June 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2025.2s.2

organic cation transporter2 (OCT2) substrate interactions. The toxicity of drugs was predicted through the study of AMES toxicity, hepatotoxicity, hERG inhibition, and rat acute and chronic toxicity predictions. These in-silico ADMET studies on these antidiabetic drug provided information about their pharmacokinetics and potential toxicity for safe and effective therapeutic use.

Results and Discussion

Quality by Design based method development

Choosing the right column is important successful separation of the analytes. Due to it's application in RP-HPLC and compatibility with moderately polar to nonpolar analytes, a column C18 was chosen for this study. This column gave good R_S and R_T for both GPR and LGL. Since longer columns are known to provide better separation, a 25 cm column was chosen to provide good R_S and R_T. To achieve good performance and column life, a 5 µm particle size was employed. Reducing particle size, increases pressure with risk of column damage but causes appreciation in R_S and efficiency. AA_{BUFFER} was chosen as the mobile phase since it is compatible with both HPLC and LC-MS instrumentations. Its volatile nature helps in compatibility with mass spectrometry detector, and buffering capacity gives a stable pH, which is essential in achieving consistent R_T and good peak shapes. A column oven was used to improve analyte performance and to lower the viscosity of mobile phase. This setup helps in decreasing the back pressure and increases the efficiency of the column. The composition of the mobile phase was optimized to provide fast and efficient separation of the eluents. Several mobile phases and ratios, such as acetonitrile, methanol, and their mixtures, were tried to meet system suitability requirements. Increasing the organic phase content in the mobile phase was found to increase the retention time of LGL and GPR when different ratios of ACN and 10 mM AA_{BUFFER} were used. The ACN: 10 mM AA_{BUFFER} ratio was the good choice, with better performance in all system suitability parameters. On the other hand, reduction in the organic content of the mobile phase resulted in broader peaks for LGL and GPR, which is likely due to the reduction in theoretical plates (N). The mobile phase was also utilized as diluent during sample preparation to prevent solvent interference during analysis. Based on the initial studies and system suitability parameters, the ACN: AA_{BUFFER} of 10 mM composition was found to be optimum. Initial experiments indicated that ACN_{Vol}, F_R, and C_T has influence on system suitability parameters, such as the N and R_S. For the optimization of chromatographic conditions, a CCD studies were adopted, taking into account the impact of these factors on significant parameters: N for LGL (R1), N for GPR (R2), and R_S (R3). Table 2 provides the data of the ANOVA evaluation that was conducted to validate the model in the Design Expert software. Quadratic models were selected for R1, R2, and R3 based on sequential p-values and residual p-values indicating a lack of fit. The model was deemed significant if the sequential p-value was less than 0.05. High corelationof data between the experimental results and the fitted models was indicated by the low coefficient of variation and high adjusted R-

Table 2: Analysis of Variance for quadratic models

Response	Std. deviation	Mean	%C.V	R^2	R^2 Adjusted	R^2 Predicted	Adequate Precision	Sequential p value	Lack of fit p value
1R	1731.84	6969	24.85	0.9998	0.9998	0.9996	11.39	<0.0001	0.3017
2R	1517.72	6215	24.42	1.0000	1.0000	0.9998	324.17	<0.0001	0.1618
3R	0.9105	5.52	16.50	0.9868	0.9697	0.9331	25.34	<0.0001	0.2627

Vol. 19 (Supplementry Issue 1A). June 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2024.3s.1

squared values. The reliability of the model was also confirmed by the observation that the adjusted R-squared and predicted R-squared values were in close equivalence for all response parameters. Depending on particular levels of each variable, the response can be predicted using the final equation. *N* of GPR (R1) = 9,272.08 + 3,583.3 * A + 783.2 * B + 3,200.8 * C + 132.125 * AB + 1,854.13 * AC + 69.375 * BC - 1,812.15 * A^2 + 620.352 * B^2 - 2,723.65 * C^2 , *N* of LGL (R2) = 8,239.3 + 3,503.1 * A + 512 * B + 3,167 * C + 45.625 * AB + 2,099.37 * AC + 80.875 * BC - 1,151.77 * A^2 + 228.732 * B^2 - 2,518.27 * C^2, R_S(R3) = 4.28873 - 5.196 * A - 0.332 * B - 3.204 * C + 0.515 * AB + 1.9425 * AC - 0.065 * BC + 1.40972 * A^2 - 1.43028 * B^2 + 2.10972 * C^2. The 2D surface plots and 3D contour plots (Figures 2 and Figure 3) helps in visualizing the interaction between factors and their effects on the responses. A quadratic relationship between the variables and the results was indicated by the curvature seen in these plots.

Design Space

The responses R1 and R2, representing the *N* for LGL and GPR respectively, the target is set at a minimum of 5000 each, while the desired R_S ranges within 3 and 5. Figure 4 illustrates the design space derived from the CCD studies meeting the specifications.

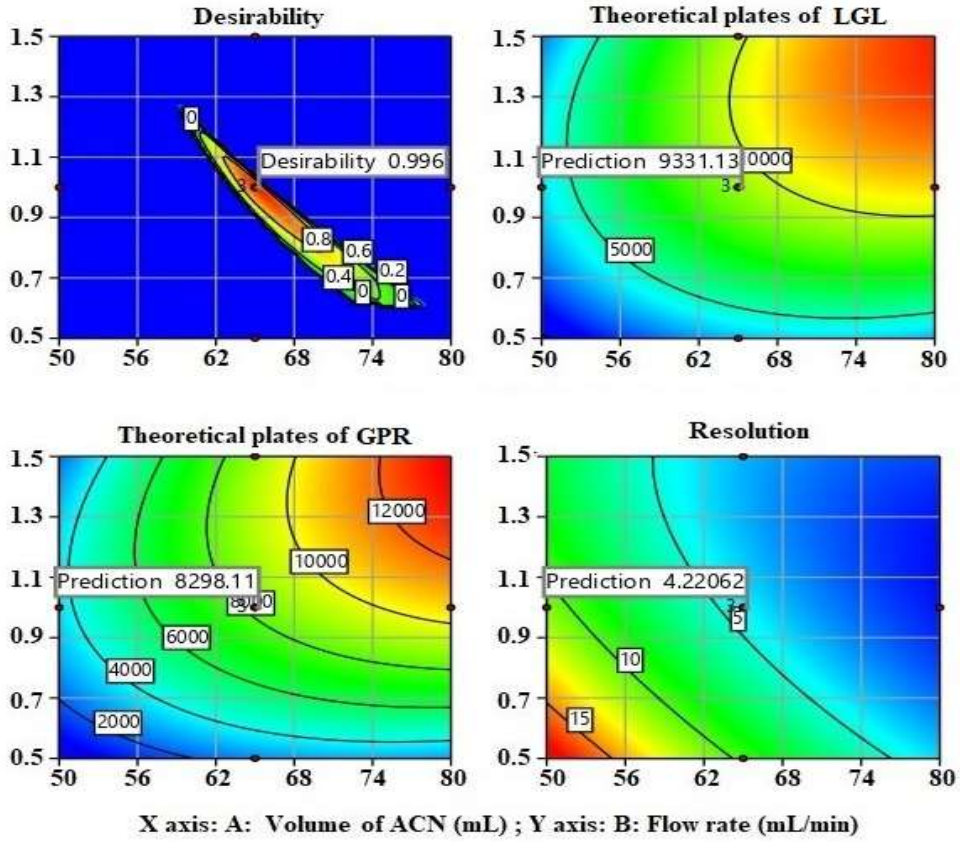

Figure 2: 2D graphs showcasing desirability and responses

Vol. 19 (Supplementry Issue 1A), June 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2025.2s.2

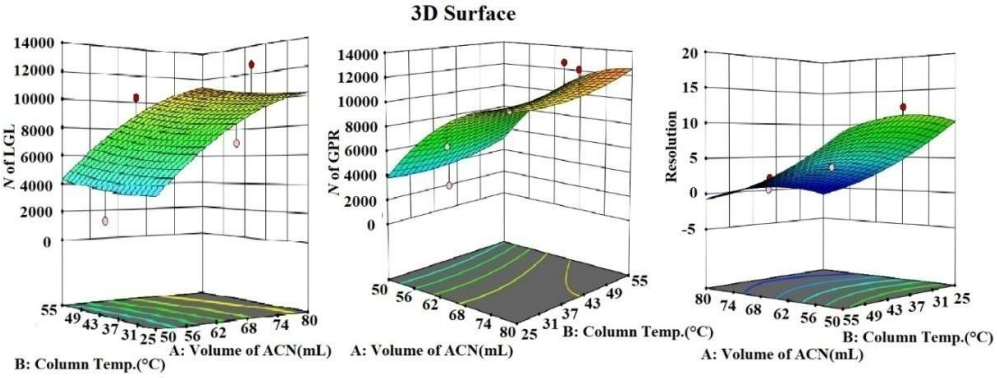

Figure 3: 3D Contour plots illustrating the impact of C_T and volume on the theoretical plates and resolution

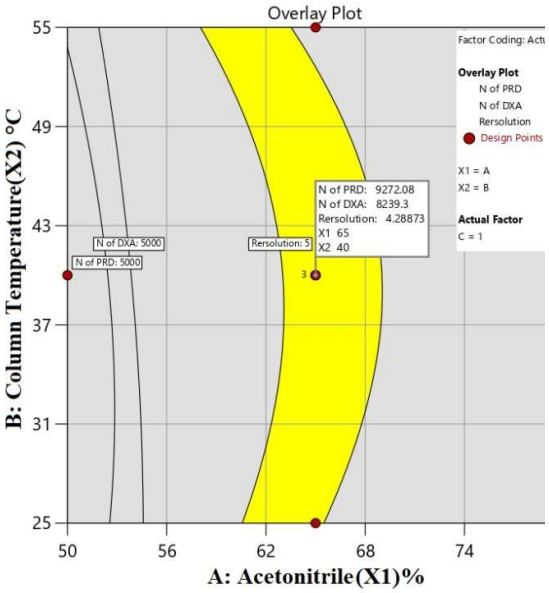

Figure 4: Design region for a CCD experiment

Optimized method

The DOE analysis determined that a mobile phase with 65% ACN, 1 mL/min of F_R, and a 40 °C C_T of were the ideal chromatographic conditions.The design space for these optimized conditions provides flexibility, with acceptable ranges of 58% to 68% ACN, 25 °C to 40 °C for C_T, and 0.7 to 1.1 mL/min for F_R. It was demonstrated that the selected parameters did not significantly impact the responses within these ranges. This confirms that the optimized method parameters are between the Method Operable Design Region (MODR), assuring the developed procedure's dependability and quality. Table 3 summarizes the experimental and predicted response values and the associated error margins, and Figure 5 shows the chromatogram that was produced.

Method Validation

Based on the UV spectra observations, GPR and LGL exhibited strong absorbance at 235 nm, making it the preferred analytical wavelength for detection. The optimized method was validated following the guidelines specified in ICH Q2 (R1). The results of the validation tests are detailed in Table 4.

Instrument Suitability Test

The optimized chromatographic conditions were evaluated for the instrument suitability test and found to be within the specification limits, confirming the methods applicability for analysis.

Linearity and Range

Regression studies for GPR and LGL were studied from 6.25–300 µg/mL and 6.25–150 µg/mL respectively. Linear curves indicated a strong linear correlation within the

Vol. 19 (Supplementry Issue 1A). June 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2024.3s.1

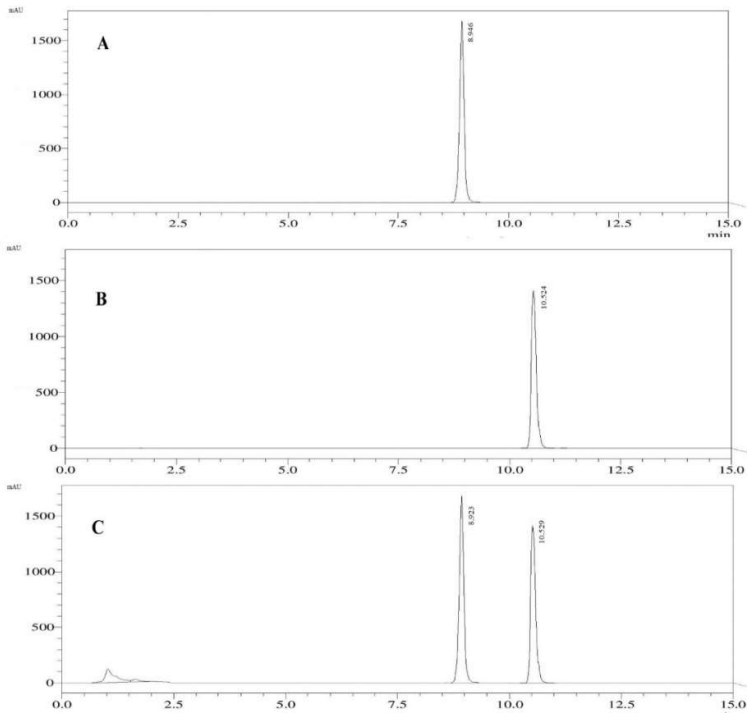

Figure 5: Chromatogram (A) standard solution of GPR, (B) standard solution of LGL and (C) sample formulation of GPR and LGL

Table 3: Experimental value compared to predicted value			
Response	Experimental mean	Predicted mean	Error(%)
1R	9172	9272	+1.09
2R	8160	8239	+0.96
3R	3.81	4.28	+12.3

Table 4: Results of validation studies		
Parameters	GPR	LGL
Linearity		
Range (µg/mL)	6.25 - 300	6.25 - 150
Regression equation	y = 152314x + 114280	y = 157895x - 131081
R^2	0.998	0.999
Precision		
Intermediate (%RSD)	1.09 – 1.52	0.98 – 1.24
Repeatability(%RSD)	0.96 - 1.34	0.87 – 1.31
Accuracy (n=3)		
50%	99.31%	99.41%
100%	99.52%	99.67%
150%	99.79%	99.74%

Vol. 19 (Supplementry Issue 1A), June 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2025.2s.2

Table 5: Robustness results

Parameter name	R_T (min)		R_S
	GPR	LGL	
As such conditions	8.92	11.53	3.85
F_R (0.9 ml/min)	9.01	11.68	4.15
F_R (1.1 ml/min)	8.78	11.39	3.69
C_T (35 °C)	9.12	11.71	4.23
C_T (45 °C)	8.68	11.36	3.66
pH of AA_{BUFFER} 3.3	8.71	11.46	3.72
pH of AA_{BUFFER} 3.7	8.97	11.61	3.96
Organic (+10%)	8.53	11.24	3.68
Organic (-10%)	9.18	11.86	3.97

Table 6: Stability data of formulation solutions for GPR and LGL

Time(h)	Assay(%)		%Difference	
	GPR	LGL	GPR	LGL
Initial	100.6	99.94	---	----
After 12h	100.1	99.89	-0.50	-0.05
After 24h	99.85	99.58	-0.75	-0.36
After 36h	99.14	98.98	-0.96	-0.96
After 48h	98.28	98.08	-1.81	-1.86

specified ranges. These calibration linearity ranges are suitable for accurately quantifying LGL and GPR in drug product.

Precision

Precision was conducted using 3 replicates at 3 distinct concentrations on variable days (50, 100, and 150 µg/mL for LGL; 25, 50, and 75 µg/mL for GPR). The % RSD values were calculated and found to be below 2% for both repeatability and intermediate precision, indicating precision of the method.

Accuracy

The recovery studies was conducted at three levels 50%, 100%, and 150% using the standard addition technique to determine the method's accuracy. The percentage recoveries for LGL and GPR were studied and were within the specification. Triplicate analyses were performed for each concentration level. The recovery results showed that the rates for both LGL and GPR ranged from 99.31% to 99.81%.

Limit of Quantification (LOQ) and Limit of Detection (LOD)

The LOQ and LOD for GPR and LGL were determined using the standard deviation method. These were calculated based on the standard deviation of the response (σ) and the slope of the calibration curve. The results showed that the LOQ and LOD for GPR were 5.39 and 1.77 µg/mL respectively, while for LGL, they were 5.05 and 1.66 µg/mL.

Robustness

To evaluate the robustness of the method, experimental parameters such as F_R, AA_{BUFFER} pH, C_T, and the proportion of the organic phase in the mobile phase were intentionally varied. The results, summarized in Table 5, remained within acceptable limits, confirming the method's robustness.

Stability data

The drug product stability was determined at five points: initial, 12, 24, 36, 48 hours, as shown in Table 6. The drug

Vol. 19 (Supplementry Issue 1A). June 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2024.3s.1

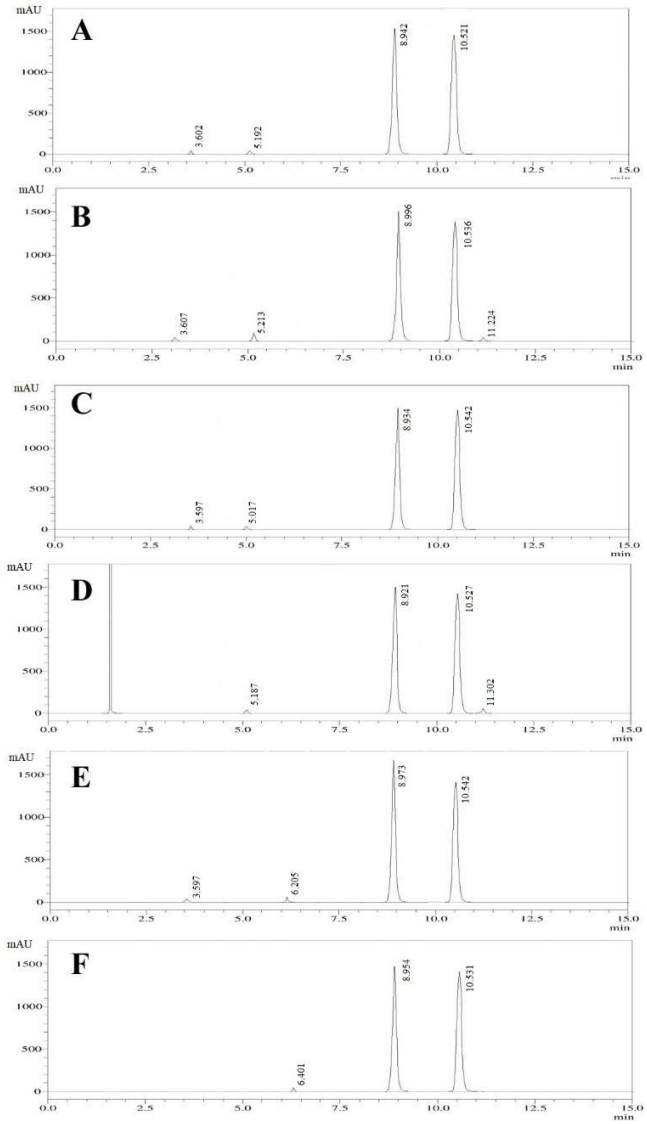

Figure 6: Chromatograms for stress degradation under the following conditions: (A) Acidic stress, (B) Basic stress, (C) Neutral stress, D) Oxidative stress, E) Photolytic stress, and F) Thermal stress

product stability samples has shown no remarkable changes, with all the results being within acceptable ranges and % RSD being less than 2%.

Forced degradation study

The drug product degradation was conducted under different stress conditions. The data results, are presented in Figure 6, shows that acid degradation resulted in 7.76% degradation for GPR, whereas LGL indicated 10.76% degradation. 8.33% of GPR and 11.31% of LGL degraded in alkaline. 8.19% degradation was observed for GPR and 8.32% for LGL in neutral. Oxidative stress

Vol. 19 (Supplementry Issue 1A), June 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2025.2s.2

Table 7: Stress degradation data

Stress condition	Assay(%)		Mass Balance		GPR		LGL	
	GPR	LGL	GPR	LGL	Purity Angle	Purity Threshold	Purity Angle	Purity Threshold
Acidic/0.01N HCl/65°C/12 h	92.24	89.24	99.14	99.27	0.144	0.359	0.155	0.348
Basic/0.01 N NaOH/ Ambient/72 h	91.67	88.69	98.92	99.39	0.136	0.338	0.143	0.311
Neutral/ 75°C/30 h	91.81	91.68	99.23	98.94	0.141	0.346	0.152	0.340
Oxidative/3%H_2O_2/72 h	92.68	91.82	99.63	98.73	0.134	0.338	0.148	0.324
Photolysis/UV lamp/72 h	93.43	94.76	99.27	99.21	0.139	0.327	0.142	0.355
Thermal/65°C/72 h	92.41	98.76	98.62	99.33	0.145	0.336	0.138	0.337

Table 8: Analysis of GPR and LGL in drug product

Drug Product	Label amount (mg)		Amount found (mg)		Recovery% ± RSD%	
	GPR	LGL	GPR	LGL	GPR	LGL
LOBG-G1 TABLETS	1.000	0.500	0.994	0.497	99.4±0.16	99.4±0.25

resulted in 7.32% degradation of GPR and 8.18% degradation of LGL. Photolytic stress tests revealed 6.57% degradation for GPR and LGL% degradation for GPR, whereas thermal stress revealed 7.59% degradation for GPR and no appreciable degradation for LGL. Complete degradation data are shown in Table 7.

Assay of drug product

The developed method was used to analyse a tablet formulation with LGL and GPR. The %mean results agreed well with the claimed label content for LGL and GPR. The results were shown in Table 8.

Comparative in-silico ADMET analysis

The *in-silico* ADMET comparative analysis on GPR and LGL revealed pharmacokinetic and safety data presented in Table 7 The absorption data for the GPR showed higher water solubility (-4.362 log mol/L) and lower intestinal absorption (64.645%), while LGL showed lower solubility (-5.518 log mol/L) and significantly higher absorption (93.003%). The LGL (0.907 log Papp) has more $CaCO_2$ permeability than the GPL (0.592 log Papp), indicating superior intestinal permeability. GPR acts an P-glycoprotein (P-gp) substrate indicating lower intracellular concentrations and potentially leading to drug resistance due to efflux whereas LGL does not has affinity P-glycoprotein leading to higher bioavailability and improved therapeutic efficiency. In distribution, Volume of distribution at steady state (VDss) for GPR (-0.339 log L/kg) is slightly higher than the LGL (-0.479 log L/kg). The negative values indicate moderate penetration into the tissues. The slightly higher VDss for GPR has advantage of

Vol. 19 (Supplementry Issue 1A). June 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2024.3s.1

slightly longer duration of action than LGL. The blood brain barrier(*BBB*) values for GPR (-0.978 log BB) and LGL (-1.466 log BB) indicates the lower penetration of LGL than GPR into BBB and less likely effects to cause CNS related side effects like dizziness in elderly people. GPR (Fu = 0.22) has a significantly more free unbound drug in the plasma than LGL (Fu=0), which is completely bound to plasma proteins. The co-administration of drugs like NSAID's, phenytoin, warfarin causes increased concentrations of GPR due to displacement reactions leading to potential toxicity. GPR has a shorter half life as the drug is more available for metabolism and excreted more quickly than LGL. In metabolism parameter, GPR and LGL have its effect on CYP3A4 as substrate and CYP2C9 as inhibitor. In addition LGL also has effect on CYP2C19 and CYP3A4 as inhibitor, indicating a complex metabolic profile with a risk of drug-drug interactions. The CYP3A4 inhibiting drugs such as ketoconazole, grape juice increase the plasma concentrations of GPR and LGL leading to toxicity. The CYP3A4 inducing drugs such as rifampin decrease the effectiveness of GPR and LGL by increasing their metabolism. The antidiabetic drugs belonging to sulfonylurea rely mainly on CYP2C9 for metabolism. The co-administration of sulfonylureas with these drugs, leads to enhanced drug effects or toxicity. The drugs belonging to proton pump inhibitors and antiplatelet drug clopidogrel, statins, calcium channel blockers, immunosuppresants and oral contraceptives relies on CYP2C19, CYP3A4 for metabolism. The co-administration with LGL drug formulation leads to enhanced effect or toxicity due to inhibitory nature on CYP2C19 and CYP3A4. In excretion parameter, GPR (0.691 log ml/min/kg) has higher clearance rate of than LGL (0.163 log ml/min/kg). The GPR is excreted faster requiring multiple daily doses. Whereas LGL is excreted slowly leading to prolonged drug action requiring once daily dosing. GPR and LGL do not excrete through organic cation transporter 2 (OCT2) present on renal tubules. These drugs are more likely to be excreted through hepatic metabolism and biliary excretion.In toxicity assessment, Lobeglitazone identified as a hERG II inhibitor, suggesting potential cardiac risks, the patients with existing cardiac diseases are vulnerable to side effects whereas Glimepiride did not show significant cardiac toxicity. Both drugs were found to be AMES toxicity negative, indicating no mutagenic nature. However, GPR was hepatotoxic, whereas LGL was not. The LD50 value for LGL (2.448 mol/kg) was slightly more than for GPR (1.942 mol/kg) indicating more acutely toxic nature of GPR than LGL. The Lowest Observed Adverse Effect Level (LOAEL) was lower for LGL (0.442 log mg/kg_bw/day) compared to GPR (0.891 log mg/kg_bw/day), suggesting that LGL may show adverse effects during chronic use. These findings emphasize the requirement of careful clinical monitoring for the drug formulation for safe therapeutic application.

Conclusion

A sensitive, reliable, accurate, and precise HPLC analytical method, developed using the Analytical QbD approach, has been optimized for the simultaneous evaluation of LGL and GPR in tablet dosage forms. The mobile phase composition, C_T, and F_R were identified as the most significant parameters influencing the variables N and R_S through CCD. The method demonstrated excellent sensitivity for detecting and quantifying LGL and GPR at low concentrations, making it suitable for routine quality control applications. Forced degradation studies validated the method's ability to the comparative ADMET study of GPR and LGL through pharmacokinetics, helps in identification of risks, which is essential for the clinical use. Glimepiride's potential for hepatotoxicity and Lobeglitazone's possible cardiac risks due to hERG inhibition highlight the need for vigilant monitoring in patients. Additionally, their differences in absorption, distribution, metabolism, and excretion

further underscore the importance of tailoring therapeutic strategies to minimize adverse effects and optimize efficacy.

Acknowledgement

The authors express their gratitude to the management of the Vignan Institute of Pharmaceutical Technology, Visakhapatnam, A.P. for providing the necessary facilities.

Conflict of Interest

The authors report that there are no competing interests to declare.

References

1. Draeger, E. (1995). Clinical profile of glimepiride. *Diabetes research and clinical practice*, 28, S139-S146.
2. National Center for Biotechnology Information. PubChem Compound Summary for CID 3476, Glimepiride. https://pubchem.ncbi.nlm.nih.gov/compound/Glimepiride. Accessed Nov. 15, 2024.
3. Bae, J., Park, T., Kim, H., Lee, M. and Cha, B.S. (2021). Lobeglitazone: a novel thiazolidinedione for the management of type 2 diabetes mellitus. *Diabetes & Metabolism Journal,* 45(3), 326-336.
4. Dutta, D., Bhattacharya, S., Kumar, M., Datta, P.K., Mohindra, R. and Sharma, M. (2023). Efficacy and safety of novel thiazolidinedione lobeglitazone for managing type-2 diabetes a meta-analysis. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, 17(1), 102697.
5. National Center for Biotechnology Information. PubChem Compound Summary for CID 9826451, Lobeglitazone. https://pubchem.ncbi.nlm.nih.gov/compound/Lobeglitazone. Accessed Nov. 15, 2024.
6. Altinöz, S. and Tekeli, D. (2001). Analysis of glimepiride by using derivative UV spectrophotometric method. *Journal of Pharmaceutical and Biomedical Analysis*, 24(3), 507-515.
7. Rabbaa-Khabbaz, L., AbiDaoud, R., Karam-Sarkis, D., Atallah, C. and Zoghbi, A. (2005). A simple and sensitive method for determination of glimepiride in human serum by HPLC. *Journal of Liquid Chromatography & Related Technologies*, 28(20), 3255-3263.
8. Jain, R., Aishwarya, D., Wankhade, S., Anupriya, Kumarasamy, M. and Peraman, R. (2024). Identification and *in vitro* genotoxicity assessment of forced degradation products of glimepiride and glyburide using HEK cell-based COMET assay. *Biomedical Chromatography,* 38(12), e6025.
9. El, Deeb, S., Schepers, U. and Wätzig, H. (2006). Fast HPLC method for the determination of glimepiride, glibenclamide, and related substances using monolithic column and flow program. *Journal of Separation Science*, 29(11), 1571-1577.
10. Song, Y., Niu, L., Wang, D., Hu, Y. and Hou, D. (2003). Direct separation and quantitative determination of glimepiride isomers by high performance liquid chromatography. *Journal of Separation Science*, 26(17), 1595-1597.
11. Yang, D., Li, X., Fu, Y., Tao, X., Zheng, F., Yu, J., Yue, H. and Dai, Y. (2021). Metabolic study of ginsenoside Rg3 and glimepiride in type 2 diabetic rats by liquid chromatography coupled with quadrupole-Orbitrap mass spectrometry. *Rapid Communications in Mass Spectrometry,* 35(11), e9083.
12. Kim, H., Chang, K.Y., Park, C.H., Jang, M.S., Lee, J.A., Lee, H.J. and Lee, K.R. (2004). Determination of glimepiride in human plasma by LC-MS-MS and comparison of sample preparation methods for glimepiride. *Chromatographia,* 60(1), 93-98.
13. Bansal, G., Singh, M., Jindal, K.C. and Singh, S. (2008). LC–UV–PDA and LC–MS studies to characterize degradation products of glimepiride. *Journal of Pharmaceutical and Biomedical Analysis,* 48(3), 788-795.
14. Gulhane, P.D. and Jawarkar, S.G. (2023). Bioanalytical method development and validation for Determination of lobeglitazone in human plasma. *International Research Journal of Modernization in Engineering Technology and Science,* 5(05), 8103-16.
15. Lee, J.H., Woo, Y.A., Hwang, I.C., Kim, C.Y., Kim, D.D., Shim, C.K. and Chung, S.J. (2009). Quantification of CKD-501,

lobeglitazone, in rat plasma using a liquid-chromatography/tandem mass spectrometry method and its applications to pharmacokinetic studies. *Journal of Pharmaceutical and Biomedical Analysis*, 50(5), 872-877.

16. Mahajan, B., Miniyar, P., Chodankar, R. and Mahajan, A. (2024). Liquid chromatography and liquid chromatography coupled with tandem mass spectrometry studies for the identification and characterization of degradation products of lobeglitazone. *Separation Science Plus*, 7(8), e202300223.

17. Marina Juliet, A. and Thanikachalam, P.V. (2025). Development and Validation of QBD-Assisted Using Central Composite Design RP-HPLC Method for Lobeglitazone Sulfate and Glimipiride in Bulk and Its Combined Dosage Form. *Chromatographia,* 1-13.

18. Sen, A.K., Sarkar, T., Sen, D.B., Maheshwari, R.A., Zanwar, A.S. and Dumpala, R.L. (2024). Quantitative determination of lobeglitazone sulfate and glimepiride in combined tablet by robust high-performance thin layer chromatographic method. *Separation Science Plus,* 7(7), e202400059.

19. Patel, D., Dobariya, J., Pradhan, P., Patel, G. and Meshram, D. (2024). Development and Validation of UV Spectrophotometric methods for simultaneous estimation of Lobeglitazone Sulfate and Glimepiride in combined dosage form. *Drug Analytical Research*, 8(1), 62-69.

20. Koppisetty, B.R.B., Kollabathula, V.R., Challa, G.N. and Yarraguntla, S.R., (2024). Quality by Design-Assisted RP-HPLC Method for Determination of Ritonavir and Darunavir in Pharmaceutical Formulation Using Central Composite Design. *Separation Science Plus,* 7(10), e202300210.

21. Dadi, V., Medapati, S., Baratam, J.K., Tatapudi, H.K., Challa, G.N., Yarguntla, S.R. and Koppisetty, B.R.B. (2024). Quality by design assisted rp-hplc method for estimation of teriflunomide and its process impurities in drug substance. *Journal of Faculty of Pharmacy of Ankara University,* 48(3), 18-18.

22. Koppisetty, B.R.B., Prasad, Y.R., Amgoth, K.M.P., Yarraguntla, S.R., Dadı, V. and Tatapudı, H.K. (2023). Utility of quality by design approach in rp-hplc method development for quantification of lamivudine and effavirenz in combination formulation. *Journal of Faculty of Pharmacy of Ankara University*, 47(2), 625-636.

23. Koppisetty, B.R.B., Tatapudi, H.K., Dadi, V., Gayathri, P.R., Komali, P., Challa, G.N., Kollabathula, V.R. and Yarraguntla, S.R. (2023). QbD based RP-HPLC method for simultaneous determination of aemtricitabine, tenofovirdiproxilfumarate and efavirenz in tablet dosage form-an application to stability indicating assay. *Analytical Chemistry Letters,* 13(3), 267-288.

24. Vasudha, D., Naidu, C.G., Koppisetty, B.R.B., Yarraguntla, S.R. and Gullapudi, T.R. (2024). A quality by design assisted RP-HPLC method utilizing central composite design for the assay of eliglustat and its organic impurities in drug. *Analytical Chemistry Letters,* 14(5), 755-769.

25. Challa, G.N., Kunda, D.R., Mustaq, S.J.H., Marni, N., Ketha, S., Gorle, U., Jakkula, S. and Koppisetty, B.R.B. (2025). QbD assisted RP-HPLC method for determination of Pyridoxine and Doxylamine in pharmaceutical formulation using central composite design. *Journal of Applied Pharmaceutical Science,* 15(4), 072-083.

26. Kumari, P.V.K., Yarraguntla, S.R., Sharmila, M. and Gulibindala, E. (2021). Application of Box-Behnken Design for Formulation Parameters of Eslicarbazepine Tablets. *Indian Journal of Pharmaceutical Sciences*, 83(3), 575-583.

27. ICH, H. T. G. (1994). Text on Validation of Analytical Procedures. In International Conference on Harmonization, Geneva, 1-5.

28. ICH, D. R. G. (2003). Stability testing of new drug substances and products Q1A (R2). In Proceedings of the International Conference on Harmonization, Geneva.

29. Vidyadhara, S., Rao, Y.S., Ramu, A., Sasidhar, R.L. and Ramya, A.J. (2013). Method development and validation for the

Vol. 19 (Supplementry Issue 1A), June 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2025.2s.2

simultaneous estimation of cinitapride and pantoprazole in solid dosage forms by RP-HPLC. *Oriental Journal of Chemistry*, 29(3), 1213.

30. Srinivasarao, Y., Kumar, T.H., Chiranjivi, P. and Rao, K.V.(2021). Simultaneous Estimation of Solifenacin Succinate and Tamsulosin Hydrochloride in Combined Dosage Form by Using First Order Derivative Spectrophotometric Method. *Indian Journal of Pharmaceutical Sciences,* 83(2), 322-326.

31. Panda, M., Dadi, V., Yarraguntla, S.R. and Rao, V.P.K. (2023). RP-HPLC method for determination of azelnidipine and telmisartan in pharmaceutical dosage form. *Research Journal of Pharmacy and Technology,* 16(2), 509-513.

32. Swathi, S., Kumar, H.T. and Rao, P.K. (2015). Validated RP-HPLC method for simultaneous determination of rosuvastatin calcium and ezetimibe in pharmaceutical dosage form. *International Journal of Pharmacy and Pharmaceutical Sciences,* 7(4), 209-213.

33. Lakshmi, G.T., Rao, Y.S., Rao, K., Prasada, V. and Kumar, T.H. (2015). RP-HPLC method for estimation of atomoxetine hydrochloride in bulk and pharmaceutical dosage form. Research *Journal of Pharmaceutical Biological and Chemical Sciences*, 6(2), 1208-1214.

34. Koppisetty, B.R.B., Yejella, R.P., Pawar, A.K.M., Yarraguntla, S.R., Kollabathula, V.R., Dadi, V. and Naidu, C.G. (2023). Development of a validated RP-HPLC assay method for quantitative separation of Teriflunomide and its process-related impurities in bulk drugs. *Journal of Applied Pharmaceutical Science,* 13(1), 028-033.

35. Kumar, T.H., Asha, C.H. and Sankar, D.G. (2019). Estimation of amlodipine besylate and irbesartan in pharmaceutical formulation by RP-HPLC with forced degradation studies. International Journal of Applied *Pharmaceutics,* 11(3), 159-167.

36. Bhanu, M.S., Dadi, V., Rao, Y.S. and Rao, V.P.K. (2023). RP-HPLC method for quantification of bilastine and monteleukast sodium in pharmaceutical dosage form. *Research Journal of Pharmacy and Technology*, 16(3), 1079-1084.