https://africanjournalofbiomedicalresearch.com/index.php/AJBR
Afr. J. Biomed. Res. Vol. 27(3) (September 2024); 2287 - 2297
Research Article

Applying the Quality by Design (CCD) technique for Simultaneous estimation of Metformin and Remogliflozin in bulk and pharmaceutical dosage form by HPTLC method

Tummala Pruthviraj[1], T. Sudha[*]

[1] Research Scholar, Vels Institute of Science, Technology and Advance Studies (VISTAS), Pallavaram, Tamilnadu, India.
[*]Department of Chemistry and Analysis, Vels Institute of Science, Technology and Advance Studies (VISTAS), Pallavaram, Tamilnadu, India.

***Corresponding Author:** Dr. T. Sudha*, M.Pharm, PhD,
Associate professor,
Department of Pharmaceutical Chemistry and Analysis,
School of Pharmaceutical sciences,
Vels Institute of Science, Technology and Advanced Studies (VISTAS), Chennai, Tamilnadu, India.
Email: tsudha.sps@vistas.ac.in
Orchid id: 0000-0001-8821-3999

ABSTRACT
The present study examines simultaneous multiple response optimization using Derringer's desirability function for the development of an HPTLC method to detect Metformin and Remogliflozin in pharmaceutical dosage form. Central composite design (CCD) was used to optimize the chromatographic conditions for HPTLC. The independent variables used for the optimization were the methanol content in the mobile phase, wavelength and the chamber saturation time. HPTLC separation was performed on aluminium plates pre-coated with silica gel 60 F254 as the stationary phase using methanol: ethyl acetate: acetic acid (7:2.5:0.5 % v/v/v) as the mobile phase.
Quantification was achieved based on a densitometric analysis of Metformin and Remogliflozin over the concentration range of 40-65 µg/mL and 4.0-6.5 µg/mL, respectively, at 245 nm. The method yielded compact and well-resolved bands at Rf of 0.24 ± 0.04 and 0.48 ± 0.03 for Metformin and Remogliflozin, respectively.
The linear regression analysis for the calibration plots produced r^2 = 0.9997 and r^2 = 0.9996 for Metformin and Remogliflozin respectively. The precision, accuracy, ruggedness, specificity, limit of detection and limit of quantitation of the method were validated according to the ICH guidelines. The factors evaluated in the validation parameters test were determined to have an insignificant **e**ffect on the selected responses.
The results indicate that the method is suitable for the routine quality control testing of marketed tablet formulations.

Keywords: Metformin, Remogliflozin, High-performance thin layer chromatography (HPTLC), Validation, Optimization, Central Composite Design (CCD)

Authors for correspondence: E-mail Id: tsudha.sps@vistas.ac.in

Received: August 2024 Accepted: September 2024

DOI: https://doi.org/10.53555/AJBR.v27i3.5282

provided that the following statement is provided. "This article has been published in the African Journal of Biomedical Research"

INTRODUCTION

One of the main causes of death worldwide is diabetes mellitus (1–3). Therefore, improved glycemic management is necessary to lower the risk of diabetic symptoms, including cardiovascular issues, retinopathy, neuropathy, and kidney failure (4). To reach the intended treatment aim and minimize side effects, combination therapy of oral hypoglycemic medications with a variety of mechanisms of action is typically advised over monotherapy (5). For the treatment of diabetic mellitus, a novel formulation of remogliflozin etabonate and metformin HCl was recently approved. Metformin HCl (figure1) is a commonly used oral hypoglycemic medication that works in several ways, including decreasing the rate of hepatic gluconeogenesis, insulin sensitivity, and intestinal absorption of glucose by increasing the anaerobic metabolism of glucose in enterocytes and decreasing insulin sensitivity, which increases the utilization of glucose by peripheral cells (6–8).

Remogliflozin etabonate (figure 2) is a recently developed oral hypoglycemic medication that does not require insulin (9, 10). It increases the removal of sugar from the urine by blocking sodium-glucose cotransporter-2 (SGLT2), an enzyme responsible for the kidneys' reabsorption of sugar. In addition to glycemic management, SGLT-2 inhibitors have a number of positive side effects, such as reducing hemoglobin A1c levels, systolic blood pressure, and body weight. RGE is therefore more beneficial when taken in conjunction with metformin, especially for patients who need to further lower their hemoglobin A1c level due to cardiac or renal conditions (11–16).

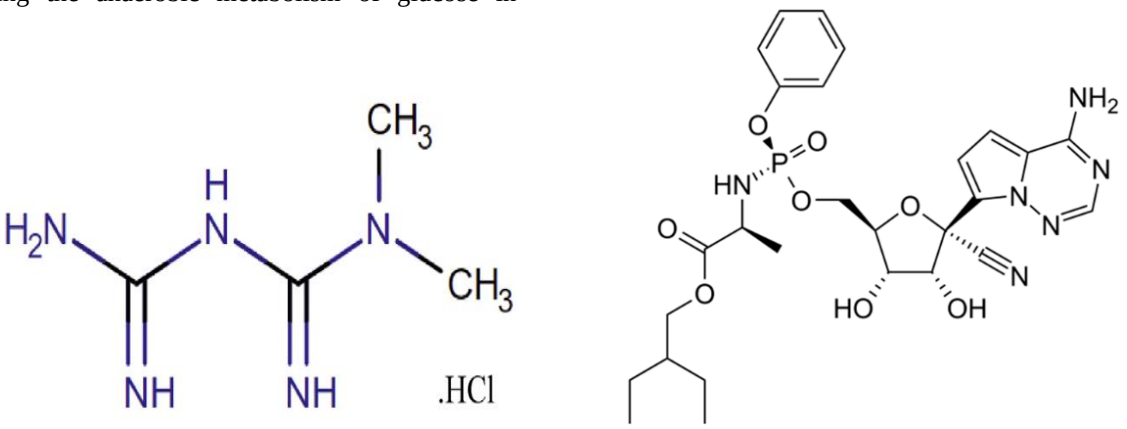

Figure 1 and 2 Structure for Metformin and Remogliflozin etabonate

Drug quantification has recently made extensive use of HPTLC due to its inexpensive maintenance costs, short analytical times, low mobile phase consumption per sample, and little sample cleanup requirements. It makes automated sample application and plate scanning possible. Additionally, it is adaptable enough to examine various sample types (17).

Literature survey states that Metformin and Remogliflozin are official in IP, USP and BP individually however, a combination of Metformin and Remogliflozin is not official in any Pharmacopoeia. Various analytical methods like HPLC (18), UV (19), stability indicating UV (20), Absorption ratio UV (21), LC-MS/MS (22), HPTLC (23), RP-UPLC (24), Mannich Reaction UV (25), Human plasma RP-HPLC (26), CCD with HPTLC (27), HPLC and HPTLC(28) Factorial Design Capillary Zone Electrophoresis (29), Multivariate optimization Capillary Zone Electrophoresis (30), for the determination of metformin alone and combined with other drugs has been reported. Similarly, UHPLC-MS/MS (31), HPLC (32), stability indicating HPTLC (33), HPTLC (34) and RP-HPLC (35) for the determination of Remogliflozin alone and combine with other drugs has been reported. Metformin combine with Remogliflozin different analytical methods like UV Derivative (36), Green UV Derivative (37), RP-HPLC (38) and UPLC (39) has been reported. Remarkably, no HPTLC method using QBD optimization for the simultaneous determination of metformin and Remogliflozin in bulk and pharmaceutical formulations has been reported in literature till date. Hence, this research paper describes the development of HPTLC method for simultaneous estimation of Metformin and Remogliflozin using Design of Experiment (DoE) approach for method validation.

DoE operates on the idea that different rational combinations of components are used to generate mathematical equations (models), graphical results, and experimental design. Pharmaceutical development, including formulation development, analytical method optimization, and validation, benefits greatly from experimental design procedures. Additionally, methodology has shown itself to be a useful tool for method validation, as it permits the investigation of concurrently changing factors. Ruggedness (various normal conditions) tests are usually conducted during method validation with the expectation that the answer will not change much, enabling the assertion of a tough technique (40). Moreover, many factors can be screened simultaneously without concerns about interacting and non-interacting effects, as they are usually considered

negligible. Various experimental designs for the study includes Plackett Burman design, factorial, fractional factorial and response surface designs.

This research article focuses on the determination of analytes by HPTLC analytical method using central composite design (CCD). Among the various experimental designs, CCD as a response surface design was preferred for prediction of nonlinear response and also due to its flexibility, in terms of experimental runs and information related to factor's main and interaction effects (41,42). Moreover, preliminary trials of optimization study revealed that the methanol content in the mobile phase produced significant effect on the response. Hence, CCD that combines two level factorial design with a star design and centre points covers the factor space near the centre with more points than at the periphery and allows more number of levels without performing experiments at every combination of factor levels. For this CCD, three influent chromatographic parameters; mobile phase composition in terms of methanol content, wavelength and saturation time on the basis of optimized experimental domain were selected and varied within a real range, and their quantitative influence on the response variable, retention factor was determined**.** Hence a novel, simple, accurate, reproducible HPTLC method was developed for simultaneous estimation of Metformin and Remogliflozin in pharmaceutical dosage form, using CCD design.

MATERIALS AND METHODS

Working standards of Metformin and Remogliflozin were kindly provided as a gift sample from Sai primus life biotech limited, Kurumbapet , Puducherry and Century Pharmaceuticals limited, Vadodara, Gujarat, India, respectively. All solvents and chemicals used were of analytical grade, purchased from Merck Specialities Pvt. Ltd., India. Marketed tablet formulations used in this study were procured from local market; Remo Zen M tablet, from Glenmark Pharmaceuticals Ltd.

Microsyringe (Linomat syringe 659.0014, Hamilton Bonaduz Schweiz, Camag, Switzerland), pre-coated silica gel 60F254 aluminium plates (10×10 cm, 100µm thickness; Merck, Darmstadt, Germany), Linomat 5 applicator (Camag, Switzerland), twin trough chamber (10×10 cm; Camag, Switzerland), UV chamber (Camag, Switzerland), TLC scanner IV (Camag, Switzerland), win CATS version 1.4.6 software (Camag, Switzerland) were used in the study. All data analysis of experimental design was performed by using the Design-Expert trial version 12.0 (Stat-Ease Inc., Minneapolis) and remaining calculations were performed by use of Microsoft Excel 2007 software (Microsoft Corporation, USA).

Chromatographic Development and scanning

The standard solutions of Metformin and Remogliflozin were spotted in the form of bands having a bandwidth of 6 mm using a Camag Linomat sample applicator a pre-coated silica gel aluminum Plate 60F254. The length of chromatographic run was 9 cm. Subsequent to the development; HPTLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed using a Camag TLC scanner with winCATS software. All measurements were made in the reflectance–absorbance mode at 245 nm, slit dimension (6.00 x 0.30 mm, micro), scanning speed 20 mm/s, and data resolution 100 µm/step. The source of radiation was a deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm.

The concentration of both drugs was determined from the intensities of diffusely reflected lights, and evaluation was done by ordinary linear regression analysis of peak areas were used as a stationary phase. Linear ascending development was carried out in a twin trough glass chamber. The mobile phase consists of Methanol: Ethyl acetate: glacial acetic acid (6:3.5:0.5 % v/v/v). The optimized chamber saturation time before chromatographic development was 30 min at room temperature (25±2 ◦C) Evaluation was via peak areas with linear regression analysis. The method was validated in accordance with ICH guidelines Q2 (R1) (43,44) for evaluation of various parameters that include linearity, precision, accuracy, LOD, LOQ, content estimation and ruggedness.

Preparation of standard stock solution

Weighed accurately about 1000mg of Metformin and 100mg of Remogliflozin were transferred into a 100ml volumetric flask and dissolved with minimum quantity of mobile phase and the volume was made up to the mark with mobile phase. Further diluted pipette out 0.55ml of this solution in to 100 ml volumetric flask and made upto the mark with mobile phase. The concentration of the solution contains 55µg/ml and 5.5µg/ml for Metformin and Remogliflozin respectively.

Preparation of Calibration Graph

The aliquots of stock solution of Metformin (0.4-0.65ml of 10000µg/ml) and (0.4-0.65ml of 1000µg/ml) for Remogliflozin were transferred in to six 100 ml volumetric flasks and made up to mark with mobile phase. The solutions containing the concentration of 40-65µg/ml for Metformin and 4-6.5µg/ml of Remogliflozin. From this solution 5µl were spotted and the chromatogram were recorded at 261nm. The above concentration range was found to be linear and obeys beer's law. The procedure was repeated for three times. The peak areas were plotted against concentration and the calibration curve was constructed.

LOD and LOQ

The linearity study was carried out for three times. The LOD and LOQ were calculated based up on the calibration curve method. The LOD and LOQ were calculated using average of slope and intercept.

Preparation of Sample solution

Twenty tablets of formulations (Remo Zen M) were weighed accurately. The average weight of the tablet

was found and powdered. The tablet powder equivalent to 1000 mg of Metformin and Remogliflozin was weighed and transferred into 100 ml volumetric flask. About 15ml of mobile phase was added to dissolve the substance. Then the solution was sonicated for 15mins. The volume was made up to required volume with the same solvent and centrifuge at 3000 rpm. Then the solution was filtered through whatmann filter paper No:41 to get 1000µg/ml Metformin of and 100µg/ml of Remogliflozin respectively. From the clear solution, further 0.55ml of this solution was diluted to 100ml with mobile phase to obtain 5.5 µg/ml of Metformin and 5µg/ml of Remogliflozin. The test solutions were spotted and recorded the chromatogram. The concentration of each test solutions was determined by using slope and intercept values from the calibration graph.

Precision

The repeatability of the method was checked by repeated analysis of the formulation for six times with the same concentrations. The amount of drug present in the formulations was calculated. The percentage RSD value was calculated.

Accuracy

To determine the accuracy of the method, recovery study was performed by standard addition method. The recovery experiment was done by adding known concentration of Metformin and Remogliflozin working standard to the pre-analysed formulations. The tablet powder equivalent to 1000mg of Metformin (100mg of Remogliflozin) was weighed accurately and transferred into a series of two 100ml standard flask. To that raw material Metformin and Remogliflozin (50, 100%,150%) were added, dissolved with 100 ml of mobile phase and made upto the volume with the same. Then the solutions were sonicated for 10 minutes. After sonication the solution was filtered through whatmann no 41. From the clear solution, pipetted out 0.55ml of test solution made into 100 ml volumetric flask and made up to mark with mobile phase. About 5µl solutions were spotted and recorded the chromatogram. Each concentration was repeated for three times.

Content Estimation (Assay)

About 55µg/ml for Metformin and 5.5µg/ml for Remogliflozin of standard and sample (Tablet formulation) solutions were prepared separately from the standard and sample stock solutions (1000µg/ml for Metformin and 100µg/ml for Remogliflozin). 5µl of each standard and sample solution were spotted and the chromatograms were recorded. The percentage purity was calculated by using peak area.

Ruggedness

The degree of reproducibility of test results by the proposed method of analytes was detected by analyzing the drug sample under following variety of test conditions. 1. Different analyst 2. Different instruments.

Forced Degradation studies

Forced degradation of each drug substances and the drug product was carried out under hydrolytic, oxidative, photolytic and thermolytic conditions. Photo degradation of drug substances and drug product was carried out in solid state. After degradation these solutions were diluted with mobile phase to achieve a concentration of 55µg/ml for Metformin and 5.5µg / ml for Remogliflozin (on label claim basis for tablet dosage form in marketed formulation). Then 5µl portions of degraded solutions by using 0.1N HCl, 0.1N NaOH,1%H_2O_2 and UV light for 24 h. were spotted and the chromatograms were recorded.

RESULTS AND DISCUSSION

Method optimization

The optimizations of chromatographic conditions were done with a view to develop HPTLC method for simultaneous determination of Metformin and Remogliflozin in bulk and in pharmaceutical dosage form.

Initial study

From the literature review, it is revealed that HPTLC method for Metformin and Remogliflozin alone or with other drug combination had been reported, where selected mobile phase comprised of methanol, ethyl acetate, acetic acid. Hence, various combinations of such components in different proportions such as methanol: ethyl acetate (6:4%v/v), methanol: acetic acid (5:5%v/v), methanol: ethyl acetate: acetic acid (8:1:1, 7: 1.5: 1.5, 6: 2: 2, 6:3.5: 0.5% v/v/v) were tried at fixed 15 min chamber saturation time and 80 mm solvent migration distance. However, satisfactory resolution of the drugs was not achieved with acceptable Rf value. Generally, chamber saturation time and solvent migration distance were crucial to HPTLC chromatographic separation. Here, chamber saturation time of less than 15 min and solvent migration distances greater than 80 mm resulted in the diffusion of the analyte band. methanol: ethyl acetate: acetic acid (6: 3.5: 0.5% v/v/v) was found to be a satisfactory mobile phase, giving good separation of Metformin and Remogliflozin.

Table 1 Chromatographic conditions optimized with CCD

			Factor A	Factor B	Factor C	Response 1	Response 2
Std	**Run**	**Space Type**	**MeOH con (%v/v)**	**Wavelength (nm)**	**Saturation time (min)**	**Rf value A**	**Rf value B**
17	1	Center	6	245	15	0.24	0.39
18	8	Center	6	245	15	0.24	0.39
16	11	Center	6	245	15	0.24	0.39
15	14	Center	6	245	15	0.24	0.39
20	17	Center	6	245	15	0.24	0.39
19	18	Center	6	245	15	0.24	0.39
14	6	Axial	6	245	18.36	0.24	0.39
13	9	Axial	6	245	11.63	0.21	0.42
12	10	Axial	6	248.36	15	0.26	0.41
11	12	Axial	6	241.63	15	0.23	0.38
9	13	Axial	4.31	245	15	0.20	0.35
10	19	Axial	7.68	245	15	0.29	0.44
4	2	Factorial	7	247	13	0.27	0.42
6	3	Factorial	7	243	17	0.24	0.40
2	4	Factorial	7	243	13	0.22	0.45
1	5	Factorial	5	243	13	0.23	0.42
7	7	Factorial	5	247	17	0.28	0.36
3	15	Factorial	5	247	13	0.25	0.37
8	16	Factorial	7	247	17	0.27	0.42
5	20	Factorial	5	243	17	0.22	0.37

But, Rf value of Metformin and Remogliflozin was found 0.24 and 0.39 was also affected by chamber saturation time. Therefore, further chromatographic conditions were optimized to obtain well-defined, compact bands of with acceptable Rf Central composite Design (CCD) is chosen due to its flexibility and can be applied to optimize HPTLC separation by gaining a better understanding of factors main and interaction effects. A three factorial, rotatable Central Composite statistical experimental design was performed using 15 experimental runs including five center points. The independent variables such as methanol content in mobile phase (A), Wavelength (B) and Saturation time (C) and the responses for all 15 optimized trial experimental runs are summarized in table 1. During model selection, it was observed that the best-fitted model for R value (<0.9) of both drugs using CCD.

Central Composite Design (CCD) was selected due to its flexibility and applied to optimize the HPTLC separation by gaining a better understanding of the factors main and interaction effects. A three-factorial, rotatable central composite statistical experimental design was employed using 15 experimental runs that included five centre points. The independent variables, such as the methanol content in mobile phase (A), Wavelength (B) and Saturation time (C), and the responses for all 15 optimized trial experimental runs are summarized in Table 1. During model selection, the best-fitted models for the Rf values of Metformin and Remogliflozin were a linear and quadratic model, respectively, based on the lowest PRESS value and adjusted R^2 value closer to 1.

Table 2 Reduced Response Surface Models and Statistical parameters obtained from ANOVA

Responses	**Regression model**	**Adjusted R^2**	**Model p value**	**C.V (%)**	**Adequate Precision**
Rf value A	+0.2397+0.0125A+0.0154B+0.0056C +0.0000AB+ 0.0000AC+0.0025BC +0.0037 A^2+0.0037 B^2-0.0033 C^2	0.9912	< 0.0001	7.30	5.5269
Rf value B	+0.3899+0.0235A-0.0014B-0.0117C+ 0.0062AB+0.0012AC+0.0112BC+0.0023A^2+0. 0023 B^2+ 0.0058 C^2	0.9015	< 0.0001	2.81	10.6485

The model was also validated with an analysis of variance (ANOVA) using the Design Expert software, and the results are presented in Table 2. Significant effects had a *P* value less than 0.0001. An adequate precision, a measure of the signal (response) to noise ratio, greater than 4 is desirable, and the obtained ratio for both drugs indicated an adequate signal (45). A coefficient of variation (% CV), which measures the reproducibility of the model, was less than 10%, and the adjusted R-square values were high, indicating a good

relationship between the experimental data and those of the fitted models. Here,the adjusted R^2 values were well within the acceptable limit of $R^2 \geq 0.80$, which indicated that the experimental data fitted polynomial equations well (46.47). The final equation, in terms of the actual components and factors, is shown in Table 2. A positive value represents an effect that favours optimization, whereas a negative value indicates an inverse relationship between the factor and the response. Three-dimensional response surface plots and perturbation plots were constructed to evaluate the effect of the factors on the retention factor of each drug. In figure 3, perturbation plots are presented for the predicted model to better understand the investigated procedure.

This figure 3 demonstrates how the response changes in response to perturbations in each factor from its defined reference value while all other factors are held constant at a reference point; the steepest slope or curvature indicates the sensitivity to a specific factor. Fig. 3(a) shows that the saturation time (factor C) had the most significant effect on the Rf value of Remogliflozin compared with other factors. Moreover, the methanol content (A) and wavelength (B) had more significant effects on the Rf value of Metformin, followed by the saturation time (factor C) (figure 3(b). Figure 4(b) response surface plots represents a variation in the Rf value of Remogliflozin as a function of the chamber saturation time and wavelength while the methanol concentration was constant.

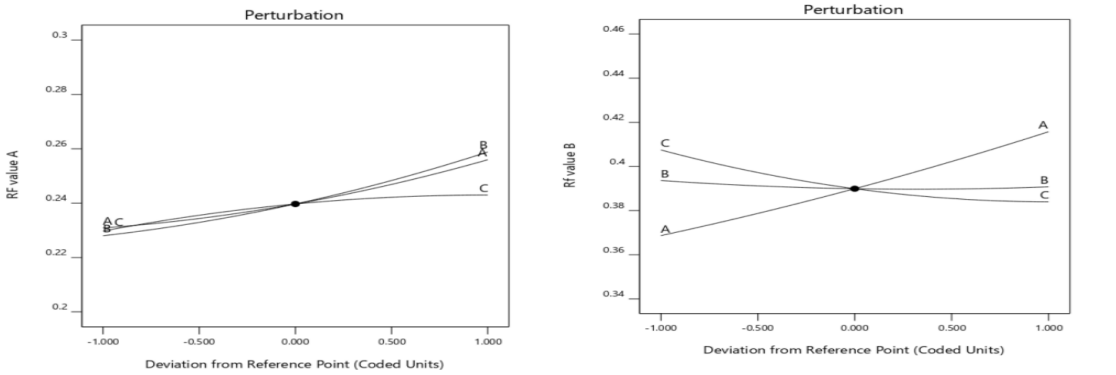

Figure 3 (a,b) Perturbation Plot for Rf value of A (Metformin) and Rf value B (Remogliflozin)

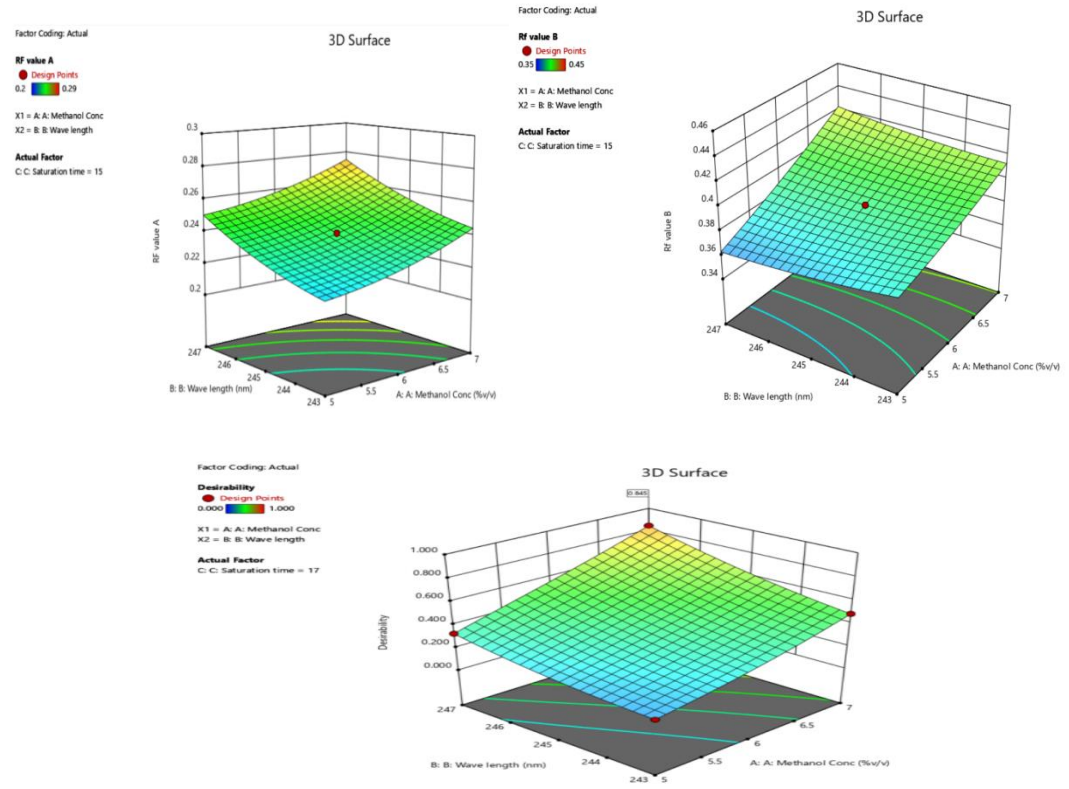

Figure 4(a,b,c) Response surface Plot for Rf value of A (Metformin), Rf value B (Remogliflozin) and Desirability plot

An analysis of the perturbation plots and response plots of the optimization model revealed that the methanol content (A) and chamber saturation time (C) more significantly affected the responses than factor B, i.e., the wavelength. The optimum conditions of separation were estimated using Derringer's desirability function. During the numerical optimization, the targets of individual factors and responses were fixed. Out of the 15 different solutions of the optimization provided by the software, two conditions that have a desirability near 0.845 were selected. The response surface obtained for the maximum Derringer's desirability function is presented in figure. 4c.

To investigate the predictability of the proposed model,the agreement between the experimental and predicted responses for both the predicted optimums, are shown in Table 3. The percentage of the prediction error was calculated using the following formula:

Predicted error = experimental-predicted/predicted × 100.

The percentage predicted error identified a set of coordinates that produced a high desirability value D = 0.845. Thus, these coordinates were used to select an optimum experimental condition to analyze Metformin and Remogliflozin in combination. The selected optimized composition for the final HPTLC analysis was methanol: ethyl acetate: acetic acid (7:2.5:0.5 % v/v/v), 17 min for chamber saturation time and 247 nm as wavelength. Under the optimized conditions, the HPTLC densitogram showed an Rf of 0.28 for metformin (50 µg/ml) and 0.42 for Remogliflozin (5 µg/ml) and is depicted in Fig. 5.

Table 3 Comparison of Experimental and Predictive values of different functions under optimal conditions

Optimum conditions	MeOH (% v/v)	Wavelength (nm)	Saturation time (Min)	Rf value A	Rf value B
Predictive	7.00	247	17	0.27	0.43
Experimental	7.00	247	17	0.28	0.42
Average error				3.70	2.32
Desirability value (D) = 0.845					

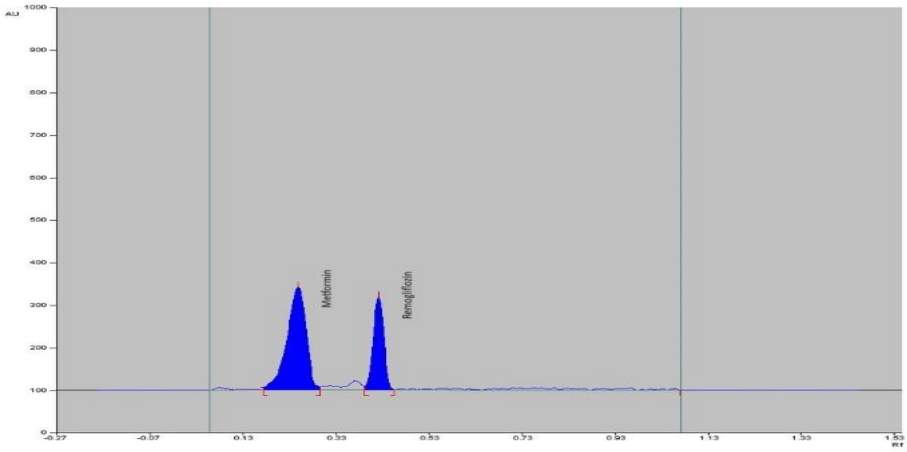

Figure 5 Chromatograms for different Experimental conditions

Method validation

The linearity of an analytical method is its ability to provide results that are directly, or via a mathematical transformation, proportional to the concentration of the analyte within a given range. The Metformin and Remogliflozin showed a good correlation coefficient (r^2 = 0.9997 for Metformin and r^2 = 0.9996 for Remogliflozin in the proposed concentration ranges of 40–65 µg/ml for Metformin and 4–6.5 µg/ml for Remogliflozin. The LOD and LOQ of the developed method were found to be 59.34 and 179.84 µg/ml, respectively, for Metformin 41.84 and 126.79 µg/ml, respectively, for Remogliflozin, indicating the sensitivity of the proposed method. The experiment was repeated for three times. The average % RSD values of metformin and remogliflozin were calculated. It was found to be less than 2%, confirming the precision of the method. When used to evaluate the recovery after spiking with three concentrations of standard, 50%, 100% and 150%, the proposed method showed percentage recovery 100.01 for metfomin and 100.96 for Remogliflozin, which were within the acceptable range of 100 ± 1%.

The analysis of the table (Remo Zen M) formulation containing 1000 mg of meformin and 100 mg of remogliflozin showed good recovery. Specifically, the percentages were 100.05% for metformin and 99.80% for remogliflozin, indicating that the method can be used for routine quality control when testing the tablet dosage formulation. The %RSD value was found to be less than 2. The developed method was validated for ruggedness. It was confirmed by using different analysts. The percentage RSD values were found to be less than 2% for two analytes. Hence the precision was further confirmed. The validation parameters reports shown in table 5.

The Stability of the drugs were confirmed by stability studies. The degradation studies was carried out by using 0.1N HCl, 0.1N NaOH, 0.1%H_2O_2 and

photolytic. Based on the results the percentage degradation was found to be for 0.1N HCl – 4.40 and 3.47 %, 0.1N NaOH-8.81 and 7.1%, 0.1% H_2O_2 – 7.87 and 9.73 %, Photolytic degradation-6.74 and 3.47 % for Metformin and Remogliflozin respectively. The degradation percentage was found to be for all stress conditions below 20 % (ICH guidelines within the limit). Hence conclude that the analytes was stable under the above stress conditions. The report was shown in table 6.

Table 5. Reports for Validation Parameters

Parameters	**Metformin**	**Remogliflozin**
Beer's law limit (µg/ mL)	40-65 µg/mL	4.0-6.5 µg/mL
Correlation coefficient (r^2)	0.9997	0.9996
Regression equation (y = mx + c)	1.5412 x + 2257.3	3.5488 x + 1577.3
Slope (m)	1.5412	3.5488
Intercept (c)	2257.3	1577.3
LOD (µg/ mL)	59.34	41.84
LOQ (µg/ mL)	179.84	126.79
Precision (% RSD)	0.1909	0.3157
Accuracy (%)	100.01	100.96
Content Estimation (Assay) (%)	100.05	99.80
Ruggedness		
Analyst-I (% RSD)	0.6170	0.8258
Analyst-II (% RSD)	0.8457	1.4307

Table 6 Data for Degradation study

Degradation Condition	**% Assay**		**% Degradation**	
	Metformin	**Remogliflozin**	**Metformin**	**Remogliflozin**
0.1N HCl Acidic / 2hr	95.60	96.53	4.40	3.47
0.1N NaOH Basic / 2hr	91.19	92.9	8.81	7.1
1% H2O2 Peroxide/2hr	92.13	90.27	7.87	9.73
Photo/ UV light/ 24hr	93.26	96.53	6.74	3.47

CONCLUSION

Essential information about the sensitivity of the Rf values of metformin and remogliflozin to different chromatographic factors can be obtained with the aid of the CCD design and response surface methods. Response surface design and Derringer's desirability function, two practical experimental design tools, were used together to optimize the methanol content, wavelength, and chamber saturation time. The results showed that a flexible process that can minimize the number of experiments required for the development and optimization of an HPTLC method is the use of a CCD design and multi-criteria decision making approaches. Additionally, it is a cost-effective technique that uses a limited number of tests to produce the most information in the shortest amount of time. The developed HPTLC method is simple, precise, and dependable, according to methodological validation. It is also appropriate for the quick quantitative measurement of metformin and remogliflozin in routine analysis. The quantities of metformin and remogliflozin in pharmaceutical dosage forms can be successfully estimated simultaneously using the suggested HPTLC approach without interference or the requirement to first separate the individual drugs.

CONFLICT OF INTEREST

The authors declared no conflict of interest

ACKNOWLEDGEMENT

The authors are thankful to the management of Vels Institute of Science, Technology and Advanced Studies, Pallavaram, Chennai, Tamilnadu, for the facilities provided to complete the Research work completely.

REFERENCES

1.Sami W, Ansari T, Butt NS, Hamid MRA. Effect of diet on type 2 diabetes mellitus: A review. International Journal of Health Sciences and Research. 2017, 11, 65–71.

2. Magliano DJ. Sacre JW, Harding JL, Gregg EW, Zimmet PZ, Shaw JE. Young-onset type 2 diabetes mellitus—Implications for morbidity and mortality. Nature Reviews Endocrinology. 2020, 16, 21–31.

3. Layla A. Anjali B. Azeem M. Prevalence of overweight, obesity, hyperglycemia, hypertension

and dyslipidemia in the Gulf: Systematic review. JRSM Short Rep. 2011, 2, 55.
4. Moon MK, Hur KY, Ko SH, Park SO, Lee BW, Kim JH, Rhee SY, Kim H, Choi KM, Kim NH. Committee of Clinical Practice Guidelines of the Korean Diabetes Association. Combination therapy of oral hypoglycemic agents in patients with type 2 diabetes mellitus. Korean Journal of. Internal Medicine. 2017, 32, 974–983.
5. Lin Y, Sun Z. Current views on type 2 diabetes. Journal of Endocrinology. 2010, 204, 1–11.
6. Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. Diabetologia 2017, 60, 1577–1585.
7. Jabbour S. Ziring B. Advantages of extended-release metformin in patients with type 2 diabetes mellitus. Postgraduate Medical Journal. 2011, 123, 15–23.
8. Setter S, Iltz J, Thams J, Campbell R. Metformin Hydrochloride in the Treatment of Type 2 Diabetes Mellitus: A Clinical Review with a Focus on Dual Therapy. Clinical Therapeutics. 2004, 25, 2991–3026.
9. Mohan V, Mithal A, Joshi SR, Aravind SR. Chowdhury S. Remogliflozin Etabonate in the Treatment of Type 2 Diabetes: Design, Development, and Place in Therapy. Drug Design Development Therapy. 2020, 14, 2487–2501.
10. Markham A. Remogliflozin Etabonate: First global approval. Drugs 2019, 79, 1157–1161.
11. Simes BC. MacGregor GG. Sodium-Glucose Cotransporter-2 (SGLT2) Inhibitors: A Clinician's Guide. Diabetes Metabolic Syndrome and Obesity. 2019, 12, 2125–2136.
12. Scheen AJ. Pharmacodynamics, efficacy and safety of sodium-glucose co-transporter type 2 (SGLT2) inhibitors for the treatment of type 2 diabetes mellitus. Drugs 2015, 75, 33–59.
13. Fujimori Y, Katsuno K, Nakashima I, Ishikawa-Takemura Y, Fujikura H, Isaji M. Remogliflozin etabonate, in a novel category of selective low-affinity sodium glucose cotransporter (SGLT2) inhibitors, exhibits antidiabetic efficacy in rodent models. Journal of Pharmacology and Experimental Therapy. 2008, 327, 268–276.
14. Dharmalingam M, Aravind SR, Thacker H, Paramesh S, Mohan B, Chawla M, Asirvatham A, Goyal R, Shembalkar J, Balamurugan R, et al. Efficacy and safety of Remogliflozin Etabonate, a new sodium glucose co-transporter-2 inhibitor, in patients with type 2 diabetes mellitus: A 24-week, randomized, double-blind, active-controlled trial. Drugs 2020, 11, 1–4.
15.Zelniker TA, Wiviott SD, Raz I, Im K, Goodrich E, Bonaca MP. SGLT2 inhibitors for primary and secondary prevention of cardiovascular and renal outcomes in type 2 diabetes: A systematic review and meta-analysis of cardiovascular outcome trials. Lancet 2019, 393, 31–39.
16. Hussey EK, Kapur A, O'Connor-Semmesm R, Tao W, Rafferty B, Polli JW, James CD, Dobbins RL. Safety, pharmacokinetics and pharmacodynamics of remogliflozin etabonate, a novel SGLT2 inhibitor, and metformin when co-administered in subjects with type 2 diabetes mellitus. BMC Pharmacolology and Toxicolohgy. 2013, 14, 25.
17. Kaul N, Agrawal H, Paradkar AR, Mahadik KR. HPTLC method for determination of nevirapine in pharmaceutical dosage form. Talanta. 2004,62, 843-52.
18. Nilesh Nikam, Avish Maru, Anil Jadhav, Prashant Malpure. "Analytical Method Development and Validation of Metformin Hydrochloride by using RP-HPLC with ICH Guidelines" Published in International Journal of Trend in Scientific Research and Development. 2019, 3(3),415-419.
19.Banothu Bhadru, Tadikonda Rama Rao, Bharath Jadav. UV Spectrophotometric Method Development and Validation for Metformin Hydrochloride in Bulk and its Tablet Formulation. Chemistry Research Journal, 2023, 8(6), 10-15.
20. Doredla Narasimha Rao, Prasada Rao M, Naga Hussain J, Lakshmi Sumanoja S, Rajeswara Rao V. Method development and validation of forced degradation studies of Metformin Hydrochloride by using UV Spectroscopy.IJPCBS 2013, 3(3), 546-553
21. Bhamare PC. A New Analytical Method Development and Validation of Metformin Hydrochloride and Fenofibrate by Absorbance Ratio UV Spectrophotometric Method. Asian Journal of Biochemical and Pharmaceutical Research. 2011, 2(1),115-128.
22.Lquadeib BT, Aloudah NM, Almurshedi AS, ALfagih IM, ALdosari BN, ALmelek AS, Almubyedh NM. Development and Validation of a Simple and Sensitive LC-MS/MS Method for Quantification of Metformin in dried blood spot its Application as an Indicator for Medication Adherence. International Journal of General Medicine, 2021, 14, 3225–3233.
23.Reddy CHM, Mubeen G, Pal M. HPTLC Method for Estimation of Metformin Hydrochloride. Biomedical and Pharmacology Journal.2008, 1(2), 123-130.
24. Cijo M Xavier, Kanakapura Basavaiah. RP-UPLC Development and validation of Metformin Hydrochloride in pure drug and Pharmaceutical formulations.World Journal of pharmacy and Pharmaceutical Sciences**.** 2015, 4(04), 1649-1668.
25. Jamil Rima, Kamil Rahme, Moussa Moussa, Mikael Rizkallaha, Karine Assakerd, Jinane K. Chabane, Frederick Naftoli Rapid Spectrophotometric Method using Mannich Reaction for Metformin Determination in Pharmaceutical tablets and human urine. International Journal of Pharmaceutical Science Review and Research. 2016, 37(2), 214-220
26.Sebaiy MM, El-Adl SM, Baraka, MM, Hassan AA. Rapid RP-HPLC method for simultaneous estimation of metformin, pioglitazone, and glimepiride in human plasma. Acta Chromatographia. 2020, 32, 16–21.
27.Munde MK, Kulkarni NS, Sen AK, Sen DB. A Novel Validated Stability Indicating Analytical Method for Simultaneous Quantification of Metformin

Hydrochloride and Empagliflozin in Bulk and Marketed Formulation by HPTLC using Box-Wilson Experimental Design Approach. Indian Journal Pharmaceutical Education and Research. 2020, 54, s644–s656.

28. Shirode AR. Maduskar PD, Deodhar MS, Kadam VJ. RP-HPLC and HPTLC Methods for Simultaneous Estimation of Metformin Hydrochloride and Vildagliptin from Bulk and Marketed Formulation: Development and Validation. British journal of pharmaceutical research. 2014, 4, 2370–2386.
29. Attimarad M. Multivariate optimization of a capillary zone electrophoresis assay method for simultaneous quantification of metformin and vildagliptin from a formulation. Journal of Liquid Chromatography & Related Technologies.2016, 39, 401–407.
30. Alnajjar AO, Idris AM. Attimarad M, Elgorashe RE. Quadruple Response Factorial Design Optimization of Capillary Zone Electrophoresis Assay Procedure for Metformin and Sitagliptin Combination. Journal of Liquid Chromatography & Related Technologies. 2015, 38, 1379–1383.
31. Smit J. Patel, Bindiya Chauhan, Basheer Shaikh, Priyanka Chavan, Nadeem Khan. Development and Validation of Selective and Sensitive Liquid Chromatography - Tandem Mass Spectroscopy (UHPLC-MS/MS) Method for Bioanalysis of Remogliflozin in Rat Plasma. Research Journal of Pharmacy and Technology. 2024; 17(10):5016-2. doi: 10.52711/0974-360X.2024.00771.
32. Shah D, Ishita I. Gondalia, Patel V, Ashok Mahajan, Chhalotiya U, Nagda D. Stability indicating thin-layer chromatographic method for estimation of antidiabetic drug Remogliflozin etabonate. Future Journal of pharmaceutical Sciences, 2021, 7, DOI:10.1186/s43094-021-00230-6.
33. Dillan Kumar, Sreenivas Rao T, Chandanam Sreedhar, Kumari Khushboo, Harsha K. Tripathy. New Analytical Method Development and Validation of an RP-HPLC for the Determination of Remogliflozin Etabonate in Bulk and Pharmaceutical Dosage Form.International Journal of Creative Research Thoughts. 2022, 7(10), 1014-1020.
34. Sarang V. Badke, Kalyani Kakad, Malode SS. High-Performance thin-layer chromatography (Hptlc) method development and validation for determination of Remogliflozin Etabonate And Vildagliptin in bulk and its tablet formulation. International Journal of applied pharmaceutics. 2022, DOI:10.22159/ijap. 2022. v14ti.42.
35. Rani JDB, Deepti CA. Method development, validation and forced degradation studies of new RP-HPLC method for simultaneous estimation of remogliflozin and teneligliptin in pure and tablet dosage form. International Journal of Pharmaceutical Sciences Research. 2023, 14(7), 3452-61. doi: 10.13040/IJPSR.0975-8232.14(7).3452-61.
36. Mahesh Attimarad, Anroop Balachandran Nair, Sreeharsha Nagaraja, Bandar Essa Aldhubiab, Katharigatta Narayanaswamy Venugopala, Shinu Pottathil. Smart UV Derivative Spectrophotometric Methods for Simultaneous Determination of Metformin and Remogliflozin: Development, Validation and Application to the Formulation. Indian Journal of Pharmaceutical Education and Research. 2021, 55(1), S293-S302.
37. Attimarad M, Nair AB, Sreeharsha N, Al-Dhubiab BE, Venugopala KN, Shinu P. Development and Validation of Green UV Derivative Spectrophotometric Methods for Simultaneous Determination Metformin and Remogliflozin from Formulation: Evaluation of Greenness. International Journal of Environmental Research and Public Health. 2021, 18(2),448. https://doi.org/10.3390/ijerph18020448.
38. Mahesh Attimarad , Rafea Elamin Elgack Elgorashe, Rajasekaran Subramaniam, Mohammed Monirul Islam, Katharigatta N. Venugopala, Sreeharsha Nagaraja, Abdulmalek Ahmed Balgoname. Development and validation of rapid RP-HPLC and green second-derivative UV Spectroscopic Methods for simultaneous quantification of Metformin and Remogliflozin in formulation using experimental design. Separations. 2020, 7(59), 1-20.doi:10.3390/separations704005
39. Tammisetty M, Challa BR, Puttagunta SB. A novel analytical method for the simultaneous estimation of remogliflozin and metformin hydrochloride by UPLC/PDA in bulk and formulation. Application to the estimation of product traces. Turkish journal of pharmaceutical sciences. 2020, 39699.
40. Vander Heyden Y, Nijhuis A, Smeyers-Verbeke J, Vandeginste BG, Massart DL. Guidance for Robustness/Ruggedness tests in method validation. Journal of Pharmaceutical and Biomedical Analysis. 2001,24,723-53.
41. Vladimir W, Anthony F. Central composite design as a powerful optimization technique for enantioresolution of the rac-11-dihydrooracin-the principal metabolite of the potential cytostatic drug oracin. Journal of biochemical and biophysical methods. 2002,54,377-90.
42. De Beer JO, Vandenbroucke CV, Massart DL, De Spiegeleer BM. Half-fraction and full factorial designs versus central composite design for retention modeling in reversed phase ion-pair liquid chromatography. Journal of Pharmaceutical and Biomedical Analysis. 1996,14,525.
43. International Conference on Harmonization ICH. (1994) Validation of Analytical: Definition and Terminology Q2A, Switzerland, p 1-4
44. International Conference on Harmonization guidance for Industry. (1996) Q2B Text on validation of Analytical methods. Switzerland, IFPMIA, p 1-8.
45. Solanki TB, Shah PA, Patel KG. Central composite design for validation of HPTLC method for simultaneous estimation of olmesartan medoxomil, amlodipine besylate and hydrochlorothiazide in tablets, Indian Journal of Pharmaceutical Sciences. 2014, 76 179–187.

46. Mustafa G, Ahuja A, Baboota S, Ali J, Box-Behnken supported validation of stability-indicating high performance thin-layer chromatography (HPTLC) method: an application in degradation kinetic profiling of ropinirole. Saudi Pharmaceutical Journal, 2013, 21, 93–102.

47. Sree Janardhanan V, Manavalan R, Valliappan K. Chemometric technique for the optimization of chromatographic system: simultaneous HPLC determination of Rosuvastatin, Telmisartan, Ezetimibe and Atorvastatin used in combined cardiovascular therapy. Arabian Journal of Chemistry. 2012, 1–10.