RESEARCH PAPER

Box–Behnken Optimized Planar Chromatographic Method for Estimation of Antihypertensive Drugs in Pharmaceutical Formulation

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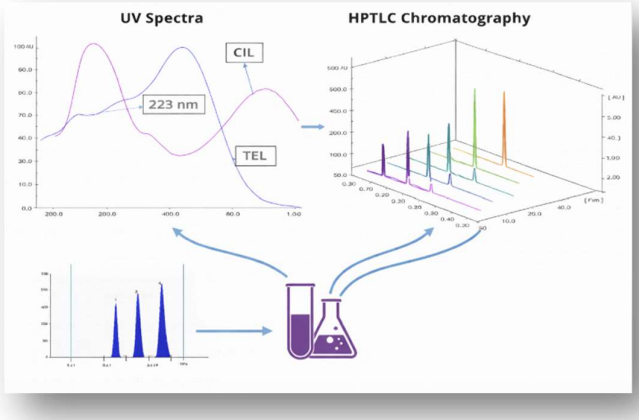
Graphical Abstract:



ABSTRACT:

Objective: To develop and validate a simple, rapid, and accurate HPTLC method for the simultaneous estimation of metoprolol succinate, cilnidipine, and telmisartan using an experimental design approach.
Method: A systematic HPTLC method was developed for the quantification of metoprolol succinate, cilnidipine, and telmisartan in tablet formulations employing a Box–Behnken design. The design was applied to evaluate the effect of critical method parameters, namely methanol volume, saturation time, and solvent front, on the Rf values of the analytes.
Result: The silica gel $60F_{254}$ pre-coated on aluminium plate was used for analyte separation as stationary phase. A mixture of toluene: methanol: chloroform: triethanolamine (7:2:1:0.1%v/v/v/v) was employed as the mobile phase. Detection was performed at 223 nm. The linearity was observed in the range of 500-2500 ng /band for metoprolol succinate, 100 - 500 ng/band for cilnidipine and 400-2000 ng/band for telmisartan as shown by $r^2 \geq 0.99$ for all three drugs. Rf value found for metoprolol succinate, cilnidipine and telmisartan was 0.26, 0.73 and 0.54 respectively. The % RSD for accuracy, precision and robustness was all within the specification, less than 2 %, which indicates that method was validated properly as per ICH Q2 (R2) guideline. The research effectively illustrates Box-Behnken design in method development was effectively used to create a chromatographic technique that is sensitive, accurate, and performs better.
Conclusion:
The developed HPTLC method is simple, rapid, accurate, and reliable for the simultaneous estimation of metoprolol succinate, cilnidipine, and telmisartan in tablet formulations. The application of Box–Behnken design

enabled efficient optimization of critical parameters, resulting in a robust and reproducible method compliant with ICH Q2 (R2) guidelines. The method is suitable for routine quality control analysis.

.**KEYWORDS:** HPTLC, Box– Behnken design, Validation, silica gel 60 F_{254}

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INTRODUCTION :

Hypertension—better known as high blood pressure—occurs when the force of blood pushing against your artery walls stays persistently elevated. Every heartbeat sends blood surging from your heart through a vast network of vessels to nourish your body. When that pressure climbs too high, your heart works overtime to keep circulation going, setting the stage for serious health risks.

According to guidelines from the World Health Organization (WHO), International Society of Hypertension (ISH), and the 7th Joint National Committee (JNC 7) on high blood pressure prevention, detection, and treatment, hypertension in adults aged 18 and older is defined as a systolic blood pressure of 140 mmHg or higher, and/or a diastolic blood pressure of 90 mmHg or higher.[2,3]

For people who have little possibility of achieving appropriate blood pressure management with monotherapy alone, combination medication is advised. By addressing several risk factors, combination drug treatment can successfully lower the risk of cardiovascular disease. For the treatment of hypertension, CDSCO has approved the combination of Metoprolol succinate ER + Cilnidipine + Telmisartan (25mg + 10mg + 40mg & 50mg + 10mg + 40mg) Tablet.

One beta blocker medication is metoprolol succinate. (Fig. 1) It is an inhibitor of beta-1-adrenergic receptors that exclusively affects heart cells; beta-2 receptors are unaffected. This inhibition reduces cardiac output without exhibiting intrinsic sympathomimetics or activity toward membrane stability because of negative chronotropic and inotropic effects. Angiotensin II receptor antagonists include telmisartan. (Figure 2) It lowers systemic vascular resistance by preventing angiotensin II from attaching to the AT1-receptor. It doesn't block ion channels or other hormone receptors. As a partial agonist of PPARγ, telmisartan improves lipid and carbohydrate metabolism and reduces insulin resistance without producing adverse effects. One dihydropyridine calcium channel blocker is cilnidipine. (Figure 3) By stopping calcium from entering the body and lessening blood vessel constriction, it decreases blood pressure. Additionally, it acts on the N-type calcium channel at the end of the sympathetic nerve, preventing the release of norepinephrine and lowering the rise in stress blood pressure. This study describes the development and validation of a high-performance thin-layer chromatography method for the simultaneous estimation of metformin HCl, cilnidipine, and telmisartan, in accordance with ICH Q2(R2) guidelines. A multivariate experimental design approach was employed to evaluate the influence of critical method parameters on analytical responses.

Multiple approaches to analysis have been reported for the estimation of individual medicines or binary combinations, according to a study of the literature. However, no analytical method has been documented for the simultaneous analysis of this specific ternary drug combination 4–21. Moreover, no publications on the use of high-performance thin-layer chromatography (HPTLC) for this combination's analysis have been reported. Nevertheless, no HPTLC method for this combination has been documented in the literature. One of the most effective experimental designs for method modelling and optimization is response surface methodology. A Box-Behnken design (BBD) was used in this investigation to maximize the chromatographic conditions. Developing a straightforward, quick, accurate, and exact HPTLC technique for quantitative analysis and validation in compliance with ICH requirements was the main goal.

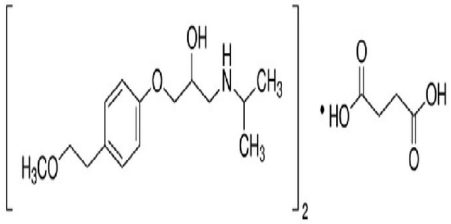

Figure 1: Structure of Metoprolol succinate

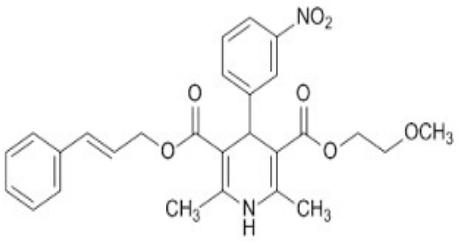

Figure 2: Structure of Cilnidipine

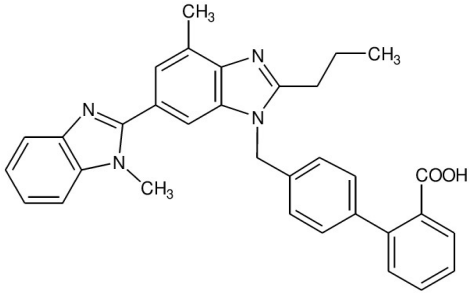

Figure 3: Structure of Telmisartan

MATERIAL AND METHODS:

API and chemicals

A free sample of metformin HCl was obtained from Emcure Pharma in Gandhinagar. A free sample of telmisartan and cilnidipine was obtained from Alembic Pharmaceutical Pvt., Ltd. in Vadodara. Merck Specialities Pvt. Ltd. in India supplied all of the analytical-grade chemicals and solvents utilized.

Instrument specifications

A CAMAG machine (Muttenz, Switzerland) was used to perform the HPTLC analysis. A Linomat V applicator fitted with a 100 µl Hamilton syringe (Bonaduz, Switzerland) was used to apply the sample. The stationary phase was made up of aluminum plates (E. Merck, Darmstadt, Germany) that had been pre-coated with silica gel 60 F_{254} purchased from Anchrom Technologists in Mumbai, India. A CAMAG TLC scanner was used to do densitometric scanning of the produced plates.

Standard solution preparation

Each of the three medications—10 mg of metformin HCl, cilnidipine, and telmisartan—was added to a individual 10 ml volumetric flask, and diluted with methanol to the appropriate level. As a consequence, standard solutions of 1000 µg/ml of metformin HCl, cilnidipine, and telmisartan were produced. To achieve working standards of 50 µg/ml metformin HCl, 10 µg/ml cilnidipine, and 40 µg telmisartan, aliquots of the stock solutions were suitably diluted with methanol.

Wavelength selection

Standard solutions of telmisartan, cilnidipine, and metformin HCl at concentrations of 1000 ng/band were applied to pre-coated HPTLC silica gel plates. The bands UV spectra were recorded by scanning them at a speed of 10 mm/s in the 200–400 nm range after chromatographic development. For further HPTLC investigation, the wavelength with the highest absorbance was chosen as the detection wavelength.

Chromatographic conditions

HPTLC plates were used in a study to assess the concentration of standard solutions. A twin trough glass chamber was used for the chromatographic development after the samples were spotted using a microsyringe on a silica gel aluminum plate 60 F254. Toluene, chloroform, methanol, and triethanolamine (7:1:2:0.1%v/v/v/v) made up the mobile phase. The chamber was saturated for 20 minutes before chromatographic development, and the run was 8 cm long. The plates were then dried using an air dryer. Densitometric scanning was performed using a Camag TLC scanner 4 with winCATS software. The measurements were made in the reflectance-absorbance mode at 223 nm, with a slit dimension of 6.00 mm × 0.30 mm (micro), scanning speed of 20 mm/s, and data resolution of 100 µm/step.

Optimization of chromatographic conditions using BBD

The method of statistical analysis for method optimization, the Box-Behnken Design (BBD) assesses the impact of several factors and their interactions. BBD efficiently captures nonlinear connections with fewer experimental runs than complete factorial designs. Its simplicity of analysis and capacity to estimate quadratic effects make it particularly advantageous [22–27]. For each *k* number of factors, the total number of experiments (*N*) in BBD is determined by 2(k−1) plus at least one center point (Co). Solvent front (A), saturation time (B), and volume of methanol (C) were recognized as essential criteria for optimization based on literature reports and early experimental findings. These factors all had a substantial impact on the medicines' Rf values. Table No. 1 displays the BBD factor and levels, whereas Table No. 2 displays the experimental domain.

Table 1: Experimental factors and levels used in BBD

Factors	Level		
	-1	0	+1
A: Solvent front (mm)	80	85	90
B: Saturation time (min)	15	20	25
C: Volume of Methanol (ml)	2	2.5	3

Experimental factors and levels used in BBD

Table No. 2, which shows a total of 17 experimental runs, illustrates the experimental domain of BBD for the specified parameters.

Table 2: Experimental domain for BBD

Run	Type	Factors		
		A: Solvent front (mm)	B: Saturation time(min)	C: Volume of Methanol (ml)

1	IBFact	80	20	2
2	Centre	85	20	2.5
3	IBFact	90	20	2
4	IBFact	85	25	2
5	Centre	85	20	2.5
6	IBFact	80	20	3
7	IBFact	85	15	2
8	IBFact	80	25	2.5
9	Center	85	20	2.5
10	IBFact	85	25	3
11	Center	85	20	2.5
12	IB Fact	80	15	2.5
13	IBFact	85	15	3
14	IBFact	90	20	3
15	IBFact	90	15	2.5
16	IB Fact	90	25	2.5
17	Center	85	20	2.5

Five duplicates of the center points were performed in order to reduce the impact of uncontrollable factors that can influence the measurements for the estimate of experimental error. All experiments were carried out in a random sequence.

Calibration curve

A working standard solution containing 1000 µg/ml of metformin HCl, cilnidipine, and telmisartan was made. A linear association between peak area and the concentration range of metformin HCl (500-2500 ng/band), cilnidipine (100-500 ng/band), and telmisartan (400-2000 ng/band) was obtained by applying various µl of these stock solutions. Five duplicate measurements were made over the concentration range of metformin HCl, cilnidipine, and telmisartan, as indicated in table no. 3 below. The concentration was expressed in ng/band.

Table 3: Calibration curve range

Sr.no	**Conc. (ng/band)**	**Applied volume**	**Conc. (ng/band) CIL**	**Applied volume**	**Conc. (ng/band) TEL**	**Applied volume**
	MET	**(µl)**		**(µl)**		**(µl)**
1	500	1	100	1	400	4
2	1000	2	200	2	800	8
3	1500	3	300	3	1200	12
4	2000	4	400	4	1600	16
5	2500	5	500	5	2000	20

ANALYTICAL METHOD VALIDATION:

The method's linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), specificity, and robustness were all verified in compliance with ICH Q2 (R2) guidelines.[28,29]

Linearity

Telmisartan, cilnidipine, and metformin HCl stock solutions with concentrations of 1000 µg/ml each were made. Various volumes of these stock solutions were used in order to get a linear connection between peak area and concentration range. Linear response was determined by analysing 5 independent levels of the concentration range of 500-2500ng/band for Metformin HCl, 100-500 ng/band for Cilnidipine and 400-2000 ng/band for telmisartan and 5 replicates were done. Although, the homoscedasticity requirement was fulfilled for regression lines, the standard deviation of slope and the intercept were calculated using ordinary least squares.

The slope of the calibration curve and the response standard deviation were used to calculate the developed method's Limit of Detection (LOD) and Limit of Quantitation (LOQ) using the formula described in the ICH recommendations.

Limit of Detection LOD = $3.3 \times \sigma/S$

Limit of Quantitation = $10 \times \sigma/S$

where "σ" = the standard deviation of the y intercepts of the regression lines,
and "S" = the slope of the calibration curve.

Precision

Three different types of experiments were conducted to determine the precision of the suggested method: repeatability, intraday precision, and interday precision. Six duplicates of the metformin HCl, cilnidipine, and telmisartan standards at 1500, 300, and 1200 ng/band, respectively, were used to measure repeatability precision in HPTLC. Both interday and intraday precision was performed on three replicates of three different concentrations for metformin HCl (500, 1500 and 2500 ng/band) cilnidipine (100, 300 and 500 ng/band) and telmisartan (400, 1200 and 2000 ng/band) in triplicate on same day and other day respectively. Peak areas were measured and

calculate percent relative standard deviation (% RSD).

By spiking standards in triplicate at three concentration levels—50%, 100%, and 150%—accuracy was assessed. Metformin HCl recovery tests were conducted by adding three different concentrations of conventional metformin HCl (500, 1000, and 1500 ng/band) to the formulation with a concentration of 1000 ng/band. Three different amounts of cilnidipine standard (100, 200, and 300 ng/band) were added to the formulation (200 ng/band). Three distinct quantities of TEL standard (400, 800, and 1200 ng/band) were added to the formulation (800 ng/band). Percent recovery determined was expressed as percent relative standard deviation (%RSD).

Specificity

The specificity of the developed HPTLC method was determined by analysing standard drug and sample solution. The band for formulation (metformin HCl: cilnidipine: telmisartan – 50:10:40 mg) was confirmed by comparing the Rf and peak purity spectra of the sample band with that of standard. Peak purity of all three drugs were assessed by comparing the spectra at three different levels, i.e., peak start (S), peak apex (M) and peak end (E) positions of the band.

Robustness

The ICH Q2 (R2) guidelines defines robustness as an analytical method's capacity to tolerate small, deliberate changes to its parameters. Variations in the various conditions taken into account for the created approach were: Mobile phase ratio-Methanol volume (2 ± 0.1 ml), saturation duration (20 ± 1 min), solvent front (80 ± 5 mm), and detection wavelength (223 ± 1 nm).

ANALYSIS OF MARKETED FORMULATION

Accurately weighed 975 mg powder equivalent to quantity of metformin HCl (50mg), cilnidipine (10mg) and telmisartan (40 mg) was taken and transferred to 50 ml volumetric flask comprising 50 ml solvent. Then, it was sonicated and filtered through whatman filter paper (Grade 42, pore size, 2 µm). The prepared solutions were used for assay by applying concentration range of 500, 1000, and 1500 ng/band for MET, 100, 200 and 300 ng/band for CIL and 400, 800 and 1200 ng/band for TEL on HPTLC plates, followed by development and scanning.

RESULT AND DISCUSSION

Selection of wavelength

For determining the appropriate wavelength, spectra of the metformin HCl, cilnidipine and telmisartan of each 10 µg/ml solution in methanol and scan in Camag TLC Scanner IV were recorded in the wavelength range 200 to 400 nm and it was found that that all three drugs showed good intensity at 223 nm. The overlay UV spectra shown in figure no. 4.

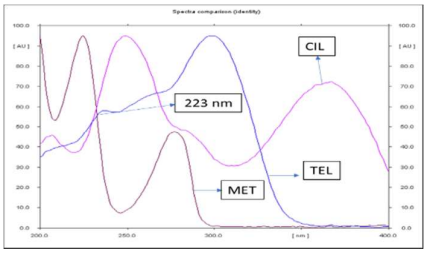

Figure. 4: UV overlay spectra of standard: metformin HCl (10 µg/ml), cilnidipine (10 µg/ml) and telmisartan (10 µg/ml) in Methanol

Optimization of chromatographic conditions using Box–Behnken Design (BBD):

Response Surface Methodology using Box–Behnken Design (BBD) was employed for efficient optimization and interaction analysis of HPTLC conditions. Based on preliminary trials, solvent front (A), saturation time (B), and methanol volume (C) were selected as key variables. A total of 17 runs, including five center points, were generated using Design Expert software. The corresponding BBD matrix and responses are provided in Table 4. Using Design Expert software version 11.1.2.0, the experimental results from different runs were input, followed by ANOVA analysis to develop the mathematical model. ANOVA was performed to identify the most significant model, with selection based on the lowest PRESS value and an R^2 closest to 1. Both linear and quadratic models showed the best fit. Key statistical parameters, including adjusted R^2, model P-value, %CV, and adequate precision, are summarized in Table 5. The adequate precision (S/N ratio) exceeded 4, confirming model significance, while a %CV below 10% indicated good repeatability and precision.

Table 4: Box-Behnken matrix showing experimental runs with responses

Run	Factor A	Factor B	Factor C	Responses		
	Solvent front (mm)	Saturation time(min)	Volume of Methanol (ml)	Rf of MET	Rf of CIL	Rf of TEL
1	80	20	2	0.25	0.73	0.59
2	85	20	2.5	0.32	0.76	0.65
3	90	20	2	0.33	0.69	0.51
4	85	25	2	0.28	0.69	0.47
5	85	20	2.5	0.31	0.75	0.64
6	80	20	3	0.51	0.78	0.68

7	85	15	2	0.25	0.69	0.53
8	80	25	2.5	0.4	0.74	0.61
9	85	20	2.5	0.31	0.74	0.63
10	85	25	3	0.51	0.88	0.77
11	85	20	2.5	0.31	0.74	0.63
12	80	15	2.5	0.34	0.71	0.6
13	85	15	3	0.4	0.81	0.69
14	90	20	3	0.53	0.86	0.75
15	90	15	2.5	0.37	0.77	0.62
16	90	25	2.5	0.42	0.82	0.69
17	85	20	2.5	0.31	0.75	0.64

Table 5: Predicted response models and statistical parameters obtained from ANOVA using BBD

Response	C.V %	PRESS	R^2	Adjusted R^2	Predicted R^2	Adequate Precision	Model
R_f of MET	3.77	0.0019	0.9895	0.9760	0.8413	26.0216	Quadratic
R_f of CIL	2.50	0.0014	0.9288	0.8861	0.7082	15.8238	Quadratic
R_f of TEL	3.70	0.0031	0.9499	0.9110	0.6720	17.6558	Quadratic

The polynomial equation representing mathematical model for various responses were shown in table no.6.

Table 6: Polynomial Equation for response

Sr. No.	Responses	Polynomial Equation
1	R_f of MET	+16.07325-0.371950*A-0.025650*B-0.145000*C-0.000100 *AB-0.00600*AC+0.008000*BC+0.002310*A^2+0.000510 *B^2 +0.141000*C^2
2	R_f of CIL	+3.21066-0.029500*A-0.30750*B-1.02750*C+0.000200*AB +0.012000*AC+0.007000*BC
3	R_f of TEL	+4.59917-0.047250*A-0.062611*B-1.35750*C+0.000600*AB +0.015000*AC+0.014000*BC-0.00522*B2

For better interpretation, the developed models are illustrated using perturbation plots (Figure 2), which show the effect of varying one factor at a time while keeping others constant. The steepness of the curve indicates the sensitivity of the response. These plots reveal that methanol volume (factor C) has the most significant influence on the Rf values of metformin HCl, cilnidipine, and telmisartan, whereas factors A and B show minimal impact. Additionally, three-dimensional response surface plots (Figure 3) were generated to depict the combined effect of two variables on the response while maintaining the third factor at a constant level.

Perturbation Plots:

The predicted models are shown as perturbation plots to enhance the understanding of the results (Figure 5). These graphs illustrate how the response varies as each factor shifts from its reference value, with all other factors kept constant. A steeper slope or curvature indicates a higher sensitivity of the response to a particular factor. Perturbation plots indicates that small variation in chamber distance travelled by solvent (A), saturation time (B) and volume of methanol (C) showed significant effect on all 3 responses as revealed from figure 5. for R_f of metformin HCl Factor A and C show minor effect, while factor B show significant effect, for R_f of cilnidipine Factor A and C show minor effect, while factor B show significant effect, and R_f of telmisartan Factor A and C show minor effect, while factor B show significant effect.

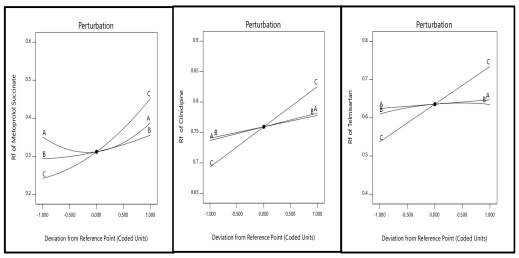

Figure 5: Perturbation graph showing the effect of each factor A, B and C on responses

Response Surface Methodology (RSM) generated three-dimensional (3D) plots (Figure 3) to illustrate the effect of two variables on the response while keeping the third constant. The plots show that increasing methanol volume leads to an increase in

the Rf values of metformin HCl, cilnidipine, and telmisartan, whereas decreasing methanol volume results in a slight reduction in their Rf values.

Three-dimensional response surface plots:

As shown in Figures 6–8, three-dimensional (3D) response surface plots were generated using RSM by varying two factors while keeping the third constant. Rf of MET: The Rf of metformin HCl increases significantly with increasing methanol volume. Rf of CIL: The Rf of cilnidipine increases notably with methanol volume, while saturation time shows minimal influence. Rf of TEL: The Rf of telmisartan is significantly affected by methanol volume, whereas saturation time and solvent front exhibit negligible effects.

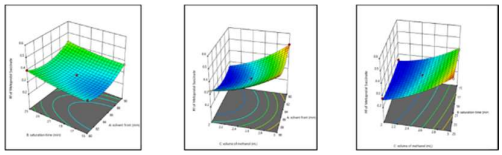

Figure 6: Three-dimensional surface plot of Rf of Metformin HCl

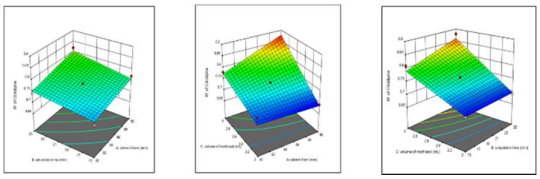

Figure 7: Three-dimensional surface plot of Rf of Cilnidipine

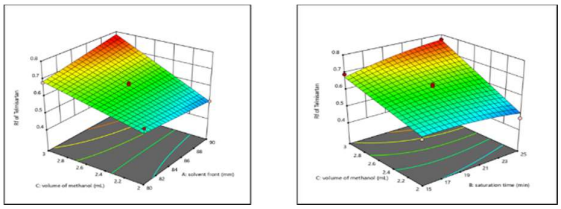

Figure 8: Three-dimensional surface plot of Rf of Telmisartan

Contour plots:

Contour plots display the relationship between two independent variables, with the magnitude of the response and other variables held constant. Figure 9-11 depicts the graphical representation of the contour plots for all 3 responses. The interpretation of contour plot is like the RSM plot as discussed in previous section.

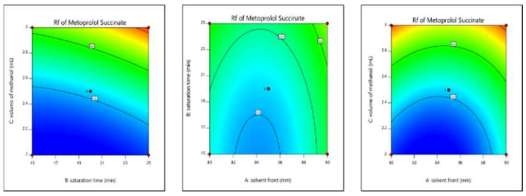

Figure 9: Contour plots of Rf of MET

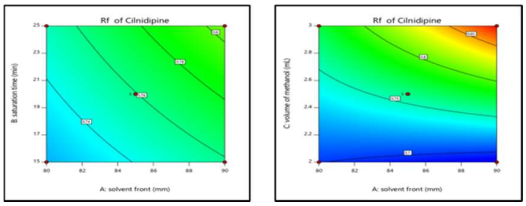

Figure 10: Contour plots of Rf of CIL

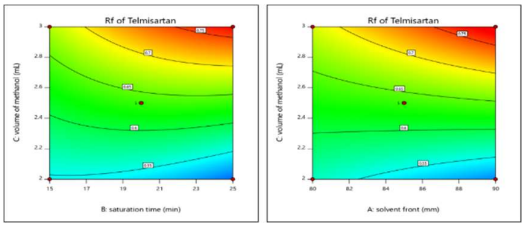

Figure 11: Contour plots of Rf of TEL

After analyzing the experimental data using ANOVA to derive the final desirability equation, the target values for each factor and response were defined using the "Numerical Optimization" function in Design Expert software. The desirability function (di), which ranges from 0 to 1, is calculated based on the previously mentioned equation. The D value approaches 1 at the global optimum, where all criteria align and are close to 1. During the optimization process, the objectives for each response and variable were specified. Two sets of conditions predicted by the software were selected for further validation of the mathematical model. The agreement between the experimental and predicted responses for the optimal conditions was then assessed to evaluate the model's predictability. The formula was applied to calculate the predicted error, using the equation:

Predicted Error = (Experimental - Predicted) / Predicted × 100

Hence the experimental conditions, Solvent front (80mm) Saturation time (20min) and volume of methanol (2 ml) was selected that gave acceptable response, and the optimized chromatogram showed Rf 0.26, 0.73 and 0.54 for Metoprolol succinate, Cilnidipine and Telmisartan at 223 nm, respectively. The analytical Design space graph in the overlay plot's yellow region indicates that the responses fall within an acceptable range, while the grey region indicates that they fall short of the intended level.

Overlay plot

In the design space graph, the yellow region represents responses within the acceptable range, while the grey region indicates responses that fall

below the desired level. Overlay plot shown in figure no.12.

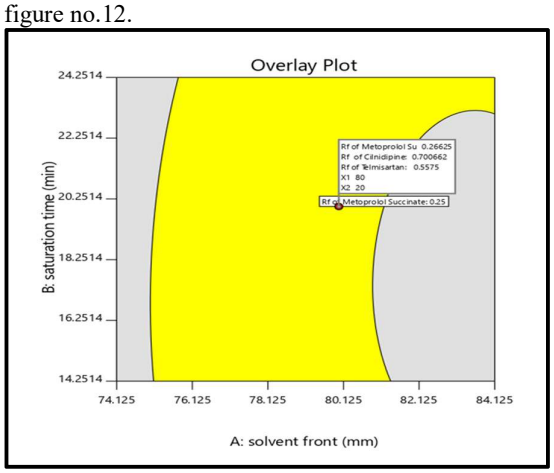

Figure 12: Overlay plot

The optimized chromatographic conditions of HPTLC method are shown in the table no.7. below.

Table 7: Optimized chromatographic conditions of HPTLC method.

HPTLC Parameters	Chromatographic conditions
Band width	8 mm
Micro-Syringe	100 µl Hamilton Syringe
Precoated Silica gel thickness	100 µm
Development	Ascending
Development Chamber	Twin trough glass chamber (10 × 20 cm)
Chamber Saturation time	20 min
Length of Chromatographic run	80 mm
Wavelength	223 nm
Mobile phase composition	Toluene: Chloroform: Methanol: TEA (7:1:2:0.1 v/v/v/v)

Figure 13: Optimized HPTLC densitogram of MET, CIL and TEL

Optimized HPTLC chromatogram showing R_f value of metformin HCl (0.26), cilnidipine (0.73) and telmisartan (0.54) at 223 nm.

ANALYTICAL METHOD VALIDATION:

Linear concentration range of 500-2500ng/band for metformin HCl, 100-500 ng/band for cilnidipine and 400-2000ng/band for telmisartan respectively (Figure 5 and Table 3). The data showed satisfactory linear relationship between the peak areas and the solution concentrations across the evaluated range.

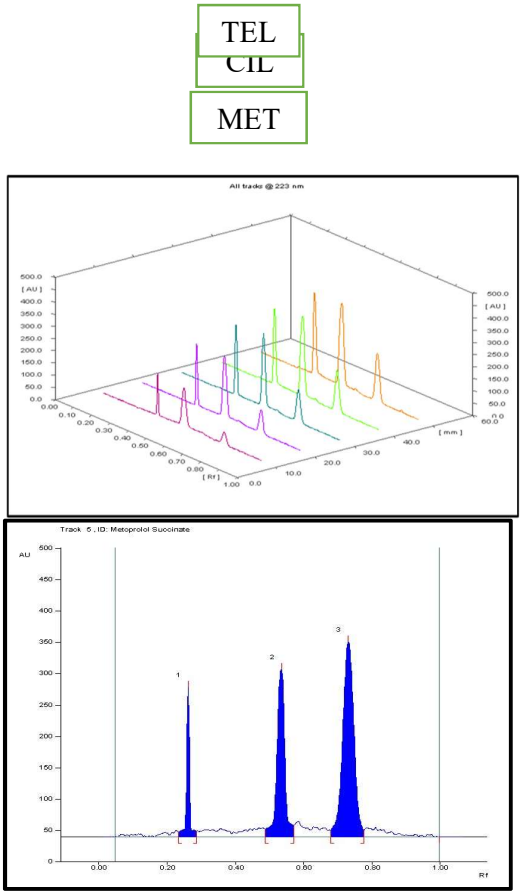

Figure 14: Chromatogram showing Linearity of HPTLC method for metformin HCl, cilnidipine and telmisartan

The LOD and LOQ values for metformin HCl, cilnidipine and telmisartan are displayed in Table no.8, demonstrating the method's sensitivity. The precision experiments pertaining to repeatability, intraday precision, and interday precision of metformin HCl, cilnidipine and telmisartan are shown in Table 8. The results indicate that the proposed approach possesses high reproducibility and repeatability, with the percentage RSD values of these studies being less than 2. After spiking metformin HCl, cilnidipine and telmisartan at known concentrations from standard solution and computing the recovery and % RSD of each drug, the accuracy of the procedure was assessed in triplicate at three concentration levels, i.e., 50 %, 100 %, and 150 %. The percentage recovery for metformin HCl, cilnidipine and telmisartan was in the range of 98.23-101.38%, 98.66% - 101.90 and 99.46% - 101.17 respectively. Where % RSD value was less than 2%; hence, the devised approach was shown to be accurate in table no.8.

Table 8 :Analysis parameters of developed HPTLC method

Parameters	MET	CIL	TEL
Calibration Range (ng/band)	500-2500	1000-5000	4000-20000
Regression Equation	y = 1.7532x + 205.88	y = 7.6455x 339.76	y = 2.4455x + 2501
Regression Coefficient (R2)	0.9954	0.9974	0.9952
SD of slope	48.4	46.14	66.58
Standard Deviation of Intercept	52.74	51.93	66.46
Limit of Detection (ng/band)	99.27	22.41	89.68
Limit of Quantification (ng/band)	300.83	67.92	271.78
Intraday precision (%RSD)	0.68	0.34	0.11
Interday precision (%RSD)	1.36	1.66	1.66
50 % (Mean% recovery ± % RSD)	98.23 ±0.22	101.90 ± 0.95	101.17 ±1.48
100 % (Mean% recovery ± % RSD)	101.38 ±0.14	99.26 ± 0.68	100.72 ±1.61
150 % (Mean% recovery ± % RSD)	98.47 ±0.14	98.66 ± 0.42	99.46 ±1.68

The robustness of the approach was shown by a small but deliberate change in numerous parameters, mobile phase composition, chamber saturation time, distance travelled by solvent and wavelength, and the impact on peak area and Rf value was measured. By comparing the retention times of metformin HCl, cilnidipine and telmisartan in chromatograms obtained from commercial formulations and standard drugs, the specificity of the method was determined. It was discovered that all the drugs had the same Rf value for both the marketed formulation and the standard drug, indicating that the method was specific (Figure no. 15,16 and 17). Furthermore, excipient interference was absent as revealed from the chromatogram of the commercial formulation.

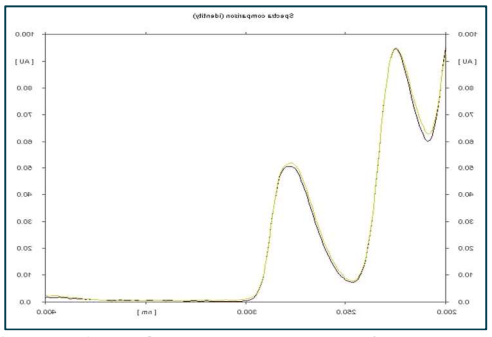

Figure 15: Overlay spectra of marketed formulation with standard metformin HCl

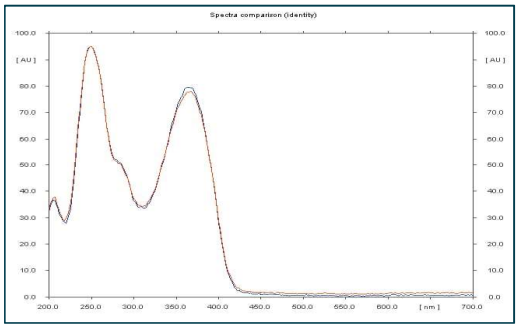

Figure 16: Overlay spectra of marketed formulation with standard cilnidipine

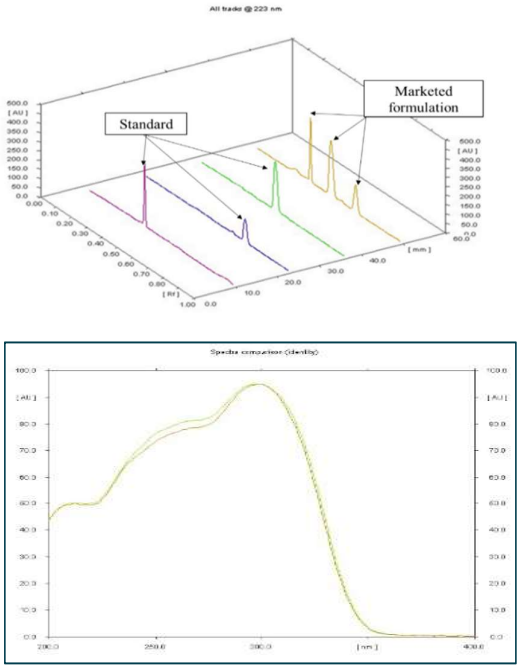

Figure 17: Overlay spectra of marketed formulation with standard telmisartan

ANALYSIS OF MARKETED FORMULATION:

The analysis of the tablet formulation containing 50 mg metformin HCl, 10mg cilnidipine and 40mg telmisartan showed good recovery and acceptable percentage amounts within the range of 98.58% - 100.56%, 100.01% - 101.79%, and 100.56% - 100.74% respectively. The HPTLC densitogram for analysis of marketed formulation are shown in figure no.18. The results are shown in table no.9.

Table 9 :Analysis of marketed formulation

Drug	Conc. (ng/band)	Conc. of drug found (ng/band)			Mean Conc. Found (ng/band)	Amount of drug (%)	SD	%RSD
		1	2	3				
METFORMIN HCl (50 mg)	500	510.50	503.65	494.31	502.82	100.56	8.12	1.61
	1000	1009.59	1017.12	1016.31	1014.34	101.43	4.13	0.40
	1500	1491.74	1477.94	1478.74	1482.80	98.85	7.74	0.52
CIL (10 mg)	100	100.73	99.73	102.76	101.07	101.07	1.54	1.52
	200	206.19	205.03	199.56	203.59	101.79	3.54	1.73
	300	299.61	303.54	296.94	300.03	100.01	3.32	1.10
TEL (40 mg)	400	402.82	406.30	397.63	402.25	100.56	4.36	1.08
	800	791.63	786.46	803.11	793.73	99.21	8.52	1.07
	1200	1231.61	1208.26	1186.96	1208.94	100.74	22.33	1.84

n =3 concentration / 3 replicate, SD = standard deviation, %RSD= relative standard deviation

Figure18: HPTLC densitogram for analysis of marketed formulation showing peak of standard metformin HCl, standard cilnidipine and standard telmisartan and their marketed formulation.

CONCLUSION:

The developed HPTLC method demonstrated satisfactory accuracy, precision, specificity, robustness, and reproducibility for the simultaneous

estimation of metformin HCl, cilnidipine, and telmisartan in marketed formulations. A notable advantage of this method is its capability to analyze multiple samples with minimal mobile phase consumption compared to HPLC, thereby reducing analysis time and overall cost while enhancing sample throughput.

To achieve optimal chromatographic conditions and evaluate the influence of critical factors on analytical response, a Box–Behnken Design (BBD) was employed. The experimental design focused on assessing the effects of methanol content in the mobile phase, solvent front, and chamber saturation time. Among these, the methanol composition significantly influenced the Rf values of metformin HCl, cilnidipine, and telmisartan, as well as the resolution between the analytes.

Furthermore, the application of BBD reduced the number of experimental trials required, ensuring an efficient optimization process. Overall, the method proved to be reliable, reproducible, and well-suited for routine quality control analysis.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation**.**

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Not applicable

AUTHOR CONTRIBUTION

Patel Foram was responsible for conceptualization, including idea generation and overall research design. Patel Gopi provided supervision, offering guidance throughout the study and approving the final manuscript. Raval Hitesh contributed to the methodology by designing the experimental protocols. Parmar Rajesh was involved in data curation, including data collection, organization, and validation. Patel Margi performed the formal analysis and interpretation of the data. Patel Dhrumi prepared the original draft of the manuscript, while Maji Dipika carried out the review and editing, ensuring critical revision and refinement of the final document.

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