FORMULATION DEVELOPMENT, OPTIMIZATION, AND STABILITY EVALUATION OF A TOPICAL COMBINATION DELIVERY SYSTEM CONTAINING DRUGS

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ABSTRACT:
The present study aimed to develop, optimize, and evaluate a topical combination gel containing Tretinoin and Mequinol for enhanced dermal delivery and improved therapeutic efficacy in the management of hyperpigmentation disorders. A Quality-by-Design (QbD) approach was employed to systematically optimize both the analytical method and formulation variables. A robust reverse-phase ultra-performance liquid chromatography (RP-UPLC) method was developed and optimized using a Box–Behnken Design (BBD) to achieve rapid and simultaneous quantification of Tretinoin and Mequinol. The optimized chromatographic conditions consisted of an ACQUITY UPLC BEH C18 column with a mobile phase comprising acetonitrile and 0.1% formic acid (70:30, v/v), delivered at a flow rate of 0.30 mL/min with detection at 254 nm. The method exhibited excellent linearity over the concentration range of 5–50 µg/mL with correlation coefficients (R^2) greater than 0.999 for both drugs. A Carbopol-based gel formulation was prepared and optimized using a Box–Behnken experimental design by evaluating the effects of Carbopol concentration, propylene glycol concentration, and pH on drug release, viscosity, and spreadability. The optimized formulation demonstrated desirable physicochemical characteristics, including viscosity of 6500–7500 cP and spreadability of 17–18 g·cm/sec. In vitro release studies revealed sustained drug release, with cumulative releases of 95.78% for Tretinoin and 89.41% for Mequinol after 8 h. Drug release kinetic analysis indicated that the release followed the Higuchi model, suggesting diffusion-controlled release from the gel matrix. The developed formulation exhibited excellent stability under accelerated storage conditions and fulfilled the predefined Quality Target Product Profile. Overall, the optimized Tretinoin–Mequinol gel represents a promising topical delivery system for effective and sustained treatment of hyperpigmentation disorders.

Keywords:
Tretinoin; Mequinol; Topical Gel; Quality by Design (QbD); Box–Behnken Design; RP-UPLC; Drug Release Kinetics; Hyperpigmentation; Carbopol Gel; Topical Drug Delivery

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INTRODUCTION:

Skin hyperpigmentation disorders, including melasma, solar lentigines, and post-inflammatory hyperpigmentation, are among the most common dermatological conditions affecting individuals worldwide. These disorders result from excessive melanin production or abnormal distribution of melanin within the epidermis and dermis, leading to cosmetic concerns and a negative impact on quality of life.[1] Topical therapy remains the preferred treatment approach because it delivers the drug directly to the affected site while minimizing systemic exposure and associated adverse effects.

Mequinol (4-hydroxyanisole) is a well-established depigmenting agent that acts by inhibiting tyrosinase activity and interfering with melanin synthesis. It has been widely used for the management of hyperpigmentation disorders due to its effectiveness and favorable safety profile.[2] Tretinoin (all-trans retinoic acid), a vitamin A derivative, promotes epidermal turnover, enhances keratinocyte differentiation, and improves the

penetration of depigmenting agents through the skin. The combination of Mequinol and Tretinoin has demonstrated superior clinical efficacy compared with monotherapy, as Tretinoin facilitates the removal of melanin-containing keratinocytes while enhancing the therapeutic activity of Mequinol.[3]

Despite their therapeutic benefits, the formulation of Tretinoin and Mequinol presents several challenges. Tretinoin is highly sensitive to light, heat, and oxidation, which can lead to degradation and reduced therapeutic effectiveness.[4] In addition, both drugs exhibit limited aqueous solubility, making it difficult to achieve uniform drug distribution and controlled release in topical dosage forms. Therefore, the development of a stable and effective semisolid delivery system is essential to maximize therapeutic performance and patient compliance.[5]

Carbopol-based gels have gained considerable attention as topical delivery systems because of their excellent bioadhesive properties, ease of application, non-greasy nature, and ability to provide controlled drug release. The incorporation of suitable solvents and penetration enhancers, such as propylene glycol, can further improve drug solubilization and dermal permeation. Optimization of formulation variables is critical to obtaining a gel with appropriate viscosity, spreadability, drug release characteristics, and long-term stability.[6]

Quality by Design (QbD) has emerged as a scientific and systematic approach for pharmaceutical product development. QbD employs risk assessment and statistical design of experiments (DoE) to identify critical formulation variables and establish their relationship with product quality attributes. Among various experimental designs, the Box–Behnken Design (BBD) is widely used for optimization studies due to its efficiency and ability to evaluate interaction effects among formulation variables while minimizing the number of experimental runs required.[7]

In the present study, a Carbopol-based topical gel containing Tretinoin and Mequinol was developed and optimized using a QbD-enabled Box–Behnken Design approach. The formulation was evaluated for critical quality attributes including drug release, viscosity, and spreadability.[8] Furthermore, an RP-UPLC method was employed for simultaneous quantification of both drugs, and the optimized formulation was assessed through in vitro release and stability studies. The study aimed to develop a stable, effective, and patient-friendly topical delivery system capable of providing controlled release of Tretinoin and Mequinol for the management of hyperpigmentation disorders.

MATERIALS AND METHODS:

Materials:

Tretinoin and Mequinol were received as gift samples from a Topclass Enterprises LLP, New delhi. Carbopol® 940, HPMC, propylene glycol, PEG 400, triethanolamine, methyl paraben, and propyl paraben were purchased from Loba Chemie Pvt. Ltd. (Mumbai, India). Acetonitrile (HPLC grade) and formic acid were obtained from Merck Life Science Pvt. Ltd. (Mumbai, India). All other solvents and reagents were of analytical grade and used without further purification. Ultrapure water generated using a Milli-Q® purification system (Merck Millipore, Bedford, MA, USA) was employed for all analytical and formulation studies.

Organoleptic Evaluation:

The physical appearance, color, odor, and texture of Tretinoin and Mequinol were visually examined to assess their organoleptic characteristics.[9]

Risk Assessment for Analytical Method Development:

A risk assessment approach was employed to identify critical method parameters (CMPs) affecting the performance of the developed UPLC method. Critical analytical attributes (CAAs), including retention time, tailing factor, and peak area, were selected as key responses. The influence of chromatographic variables such as mobile phase composition, flow rate, detection wavelength, column temperature, injection volume, and buffer pH was evaluated using a Risk Estimation Matrix (REM). Based on the risk analysis, organic phase composition and flow rate were identified as high-risk factors and selected for further optimization using a Box–Behnken experimental design. The Quality Target Product Profile (QTTP) and Analytical Target Profile (ATP) were established to ensure that the method met predefined requirements for accuracy, precision, specificity, robustness, and stability-indicating performance.[10-11]

Table 1: Risk Analysis for the Analytical Method Development

CQA (Critical Quality Attributes)	Analytical Method Risk					
	CPP (Critical Process Parameters)					
	Organic Phase (%)	Flow Rate (mL/min)	Detection Wavelength (nm)	Column Temperature (°C)	Injection Volume (µL)	Buffer pH
Retention Time (min)	High	High	Low	Medium	Low	Medium

Tailing Factor	Medium	Medium	Low	High	Medium	High
Peak Area	High	Medium	High	Low	Medium	Medium

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Table 2: Quality target product profile (QTTP) will be developed in line with the project's primary goal.

Response	Parameter	Goal
Y1	Retention Time (min)	Minimize
Y3	Tailing Factor	Minimize
Y4	Peak Area	Maximize

Analytical Method Development:

Chromatographic Conditions:

Chromatographic analysis was performed using a Waters RP-UPLC system equipped with a photodiode array (PDA) detector. Separation was achieved on an ACQUITY UPLC BEH C18 column (100 × 2.1 mm, 1.7 µm) maintained at 35°C. The mobile phase consisted of acetonitrile and 0.1% (v/v) formic acid in water (70:30, v/v) delivered in isocratic mode at a flow rate of 0.30 mL/min. The injection volume was 2 µL, and detection was carried out at 254 nm. The total run time was 5 min. The mobile phase was used as the diluent for preparation of standard and sample solutions.[12-14]

Preparation of Standard and Sample Solutions:

Standard stock solutions of Tretinoin and Mequinol (1000 µg/mL) were prepared by dissolving 10 mg of each drug in the mobile phase and diluting to 10 mL. Working standard solutions were prepared by appropriate dilution to obtain concentrations ranging from 5–50 µg/mL for calibration studies. Sample solutions were prepared by accurately weighing the formulation equivalent to 10 mg of each drug, followed by extraction, sonication, filtration, and suitable dilution with the mobile phase. All solutions were filtered through a membrane filter before analysis, and Tretinoin-containing solutions were protected from light.[15-16]

QbD-Based Optimization of RP-UPLC Method:

A Quality-by-Design (QbD) approach employing a Box–Behnken Design (BBD) was utilized to optimize the RP-UPLC method using Design-Expert® software (Version 13.0, Stat-Ease Inc., USA). The effects of three critical method parameters, namely organic phase composition (A), flow rate (B), and column temperature (C), were evaluated on chromatographic responses including retention time, tailing factor, and peak area. Each factor was investigated at three levels (−1, 0, and +1), generating 13 experimental runs. The experimental data were fitted to a quadratic polynomial model:[17-18]

$$Y = a_0 + a_1A + a_2B + a_3C + a_{12}AB + a_{13}AC + a_{23}BC + a_{11}A^2 + a_{22}B^2 + a_{33}C^2 \ldots \textit{(Eq. 1)}.$$

where *Y* represents the chromatographic response and *a* denotes the regression coefficients. Response surface plots and contour plots were generated to identify the optimal chromatographic conditions. The optimized method provided improved peak symmetry, retention behavior, and analytical sensitivity, demonstrating its suitability for routine analysis and stability studies of Tretinoin and Mequinol.

Table 3: Box–Behnken Experimental Design (Coded Levels)

Run	A (% Organic Phase)	B (Flow Rate)	C (Temperature)
1	-1	-1	0
2	+1	-1	0
3	-1	+1	0
4	+1	+1	0
5	-1	0	-1
6	+1	0	-1
7	-1	0	+1
8	+1	0	+1
9	0	-1	-1
10	0	+1	-1
11	0	-1	+1
12	0	+1	+1
13	0	0	0

Factor Code	Variable	Low (-1)	Medium (0)	High (+1)
A	% Acetonitrile (Organic Phase)	60	70	80
B	Flow Rate (mL/min)	0.25	0.30	0.35
C	Column Temperature (°C)	30	35	40

Calibration Curve Preparation and Linearity Study:

Preparation of Calibration Standards:

A working standard solution at 100 µg/mL was used to prepare a set of calibration standards. We carefully pipetted 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mL into separate 10 mL volumetric flasks, then added the appropriate diluent to each flask to obtain final concentrations of 5, 10, 20, 30, 40, and 50 µg/mL. Before being analyzed, each solution was mixed well and passed through a 0.22 µm membrane filter.[19]

Chromatographic Conditions:

We used an RP-UPLC system with an ACQUITY UPLC BEH C18 column (100 mm × 2.1 mm, 1.7 µm) for chromatographic analysis. The column temperature remained at 35 °C. The mobile phase consisted of 0.1% (v/v) formic acid in water (Solvent A) and 30% (v/v) acetonitrile, with a 30:70 (v/v) isocratic ratio. The flow rate was 0.30 mL/min, and the injection volume was 2 µL. The detection was at 254 nm, and the entire process took 5 minutes.[20-22]

Table 4: Optimized RP-UPLC Chromatographic Conditions

Parameter	Condition
Column	ACQUITY UPLC BEH C18 (100 mm × 2.1 mm, 1.7 µm)
Column Temperature	35 °C
Mobile Phase (Solvent A)	0.1% (v/v) Formic Acid in Water
Mobile Phase (Solvent B)	Acetonitrile
Elution Mode	Isocratic
Mobile Phase Composition	Acetonitrile: 0.1% Formic Acid (70:30 v/v)
Flow Rate	0.30 mL/min
Injection Volume	2 µL
Detection Wavelength	254 nm
Run Time	5 minutes

Calibration Curve Construction:

Each calibration standard solution was injected three times under the best chromatographic conditions. We recorded the analyte peak areas and generated a calibration curve by plotting the mean peak area against concentration (µg/mL).[2-24]

Table 5: Calibration Curve Preparation (Linearity range: 5–50 µg/mL)

Sr. No.	Volume Taken from Working Solution (100 µg/mL) (mL)	Final Volume (mL)	Concentration (µg/mL)
1	0.5	10	5
2	1.0	10	10
3	2.0	10	20
4	3.0	10	30
5	4.0	10	40
6	5.0	10	50

Solubility Studies:

The solubility of Tretinoin and Mequinol was investigated in different solvents, including propylene glycol, ethanol, and purified water. Excess amounts of each drug were added separately to 1 mL of the selected solvents and stirred at room temperature for 24 h to attain equilibrium. The mixtures were centrifuged at 5000 rpm for 15 min, and the supernatants were filtered. The filtered samples were suitably diluted with the mobile phase and analyzed using the developed RP-UPLC method at 254 nm. The solvent exhibiting maximum drug solubility was selected for formulation development.[25]

Risk Assessment for Gel Formulation Development:

A Quality-by-Design (QbD)-based risk assessment was performed to identify the Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs) that could influence the Critical Quality Attributes (CQAs) of the Tretinoin–Mequinol gel formulation. A Risk Estimation Matrix (REM) was employed to evaluate and classify the potential impact of formulation and process variables as high, medium, or low risk. Based on the risk assessment outcomes, critical factors affecting formulation quality were identified and selected for further optimization. The Quality Target Product Profile (QTTP) was established to define the desired quality characteristics of the final topical gel formulation.[26]

Table 6: Risk Analysis for gel formulation and development

CQA (Critical Quality Attributes)	Analytical Method Risk CPP (Critical Process Parameters) Tretinoin (%)	Mequinol (%)	Carbopol 940 (%)	Propylene Glycol (%)	Triethanolamine (pH)	Purified Water (%)
Viscosity (cP)	Low	Low	High	Medium	High	Medium
Drug Release (%)	High	High	High	High	Medium	Medium
Spreadability	Low	Low	High	Medium	Medium	High

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Table 7: Quality target product profile (QTTP) will be developed in line with the project's primary goal

Response	Parameter	Goal
Y1	Drug Release (%)	Maximize
Y3	Viscosity (cP)	Optimize
Y4	Spreadability	Maximize

Preparation of Tretinoin–Mequinol gel:

The gel formulation was prepared using a Carbopol-based gelation method. Accurately weighed Carbopol 940 was dispersed in purified water and allowed to hydrate for 30–60 minutes. Methyl paraben was dissolved in a small quantity of warm water or propylene glycol. Mequinol was dissolved in propylene glycol, followed by the addition of Tretinoin under light-protected conditions. The drug-containing solution was gradually added to the Carbopol dispersion with continuous stirring. The formulation pH was adjusted to 5.5–6.0 with triethanolamine, resulting in gel formation. The final volume was adjusted with purified water, and the mixture was homogenized to obtain a uniform gel.[27-32]

Experimental Design and Formulation Optimization:

The Tretinoin–Mequinol gel formulation was optimized using a Quality-by-Design (QbD) approach based on a Box–Behnken Design (BBD) generated with Design-Expert® software (Version 13.0, Stat-Ease Inc., USA). Three independent variables, namely Carbopol concentration (A, 0.5–1.5% w/w), propylene glycol concentration (B, 5–10% w/w), and formulation pH adjusted using triethanolamine (C, 5.5–6.5), were evaluated at three levels. The effects of these variables on drug release (Y_1), viscosity (Y_2), and spreadability (Y_3) were investigated. Experimental data were fitted to a quadratic polynomial model:[33-34]

$$Y = a_0 + a_1A + a_2B + a_3C + a_{12}AB + a_{13}AC + a_{23}BC + a_{11}A^2 + a_{22}B^2 + a_{33}C^2 \ldots \textit{(Eq. 2).}$$

where Y represents the measured response, a_0 is the intercept, a_1–a_{33} are regression coefficients, and A, B, and C represent the independent variables. Response surface and contour plots were used to identify the optimum formulation conditions.

***In Vitro* Drug Release Study:**

The in vitro release of Tretinoin and Mequinol from the developed gel was evaluated using a Franz diffusion cell fitted with a dialysis membrane (MWCO 12,000–14,000 Da). The receptor compartment contained phosphate buffer (pH 6.8) supplemented with 20% v/v ethanol and was maintained at 37 ± 0.5°C under continuous stirring at 500 rpm. An accurately weighed quantity of gel was placed in the donor compartment. Samples were withdrawn at predetermined intervals up to 8 h and replaced with fresh receptor medium to maintain sink conditions. The collected samples were filtered through a 0.22 µm membrane filter and analyzed using the validated RP-UPLC method at 254 nm. The cumulative percentage drug release was calculated using the respective calibration curves.[35-36]

Drug Release Kinetic Analysis:

The release kinetics of Tretinoin and Mequinol were evaluated by fitting the in vitro release data to various mathematical models, including zero-order, first-order, Higuchi, Hixson–Crowell, and Korsmeyer–Peppas models. The goodness of fit was assessed using the correlation coefficient (R^2), and the release mechanism was characterized by the release exponent (n) obtained from the Korsmeyer–Peppas model.[37-39]

Stability Studies:

Accelerated stability studies were conducted according to ICH guidelines. Optimized formulations were packed in suitable containers and stored at 40 ± 2°C and 75 ± 5% relative humidity for three months. Samples were withdrawn at predetermined intervals and evaluated for appearance, pH, viscosity, drug content, and in vitro drug release characteristics.[40]

Statistical Analysis:

Experimental results were expressed as mean ± standard deviation. Statistical analysis was performed using Design-Expert® software and GraphPad Prism®. Analysis of variance (ANOVA) was employed to evaluate the significance of formulation variables. A p-value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION:

Organoleptic Evaluation:

Tretinoin was observed as a yellow to light-orange crystalline powder, while Mequinol appeared as a white to off-white crystalline powder. Both drugs exhibited characteristic odor, crystalline texture, and were free from visible impurities. The observed organoleptic properties were consistent with standard specifications, confirming the suitability of both drugs for formulation development.

Table 8: Organoleptic characteristics of Tretinoin and Mequinol

Parameter	Tretinoin	Mequinol
Appearance	Yellow to light-orange crystalline powder	White to off-white crystalline powder
Odor	Characteristic	Characteristic
Texture	Crystalline powder	Crystalline powder
Purity	Free from visible impurities	Free from visible impurities

Optimization of RP-UPLC Method Using Box–Behnken Design:

A QbD-based Box–Behnken Design (BBD) was employed to optimize the RP-UPLC method by evaluating the effects of organic phase composition (% acetonitrile), flow rate, and column temperature on retention time, tailing factor, and peak area of Tretinoin and Mequinol. The experimental responses showed good agreement with the predicted values, confirming the suitability of the quadratic model for method optimization.

Statistical analysis revealed that all developed models were significant ($p < 0.05$), indicating a substantial influence of the selected chromatographic variables on method performance. Among the factors studied, the percentage of acetonitrile exhibited the greatest effect on chromatographic responses. Increasing the organic phase concentration reduced retention time and enhanced peak area for both analytes, whereas flow rate showed a moderate influence and column temperature exerted comparatively smaller effects.

Response surface and contour plots demonstrated the interactive effects of the independent variables and facilitated identification of the optimum chromatographic conditions. The predicted versus actual plots showed excellent agreement, indicating high predictive capability of the models. Furthermore, the obtained statistical parameters, including high R^2 values (>0.97) and adequate precision values (>4), confirmed the robustness and reliability of the developed method.

Optimization using the desirability function identified the optimum chromatographic conditions as 70% acetonitrile, a flow rate of 0.30 mL/min, and a column temperature of 35°C. Under these conditions, Tretinoin and Mequinol were eluted at retention times of approximately 1.22 min and 2.45 min, respectively, with acceptable tailing factors (<1.5) and high peak responses. The optimized method provided rapid separation, excellent peak symmetry, and enhanced sensitivity, demonstrating its suitability as a robust stability-indicating RP-UPLC method for simultaneous estimation of Tretinoin and Mequinol in topical formulations.

For Tretinoin, the polynomial equations derived from the quadratic model are showed in Eqs. 3−5.

Retention time (min) = $1.22 - 0.21875A - 0.085B - 0.02875C + 0.030AB + 0.0175AC + 0.000BC + 0.15875A^2 + 0.10125B^2 + 0.10375C^2$…… **(Eq. 3)**

Tailing factor = $1.30 - 0.0225A + 0.02375B + 0.02125C + 0.000AB + 0.000AC - 0.0025BC + 0.09125A^2 + 0.05875B^2 + 0.03875C^2$…… **(Eq. 4)**

Peak area (AU) = $560000 + 23125A + 8500B - 4625C + 7500AB + 3750AC + 2000BC - 21625A^2 - 20875B^2 + 5375C^2$…… **(Eq. 5)**

For Mequinol, the polynomial equations are depicted in Eqs. 6−8.

Retention time (min) = $2.45 - 0.1525A - 0.06875B - 0.02125C + 0.0375AB + 0.0075AC - 0.025BC + 0.1225A^2 + 0.065B^2 + 0.085C^2$…….. **(Eq. 6)**

Tailing factor = $1.28 - 0.01875A + 0.020B + 0.01875C + 0.040AB - 0.0025AC + 0.000BC + 0.10125A^2 + 0.07875B^2 + 0.01125C^2$…… **(Eq. 7)**

Peak area (AU) = $900000 + 29375A + 4375B - 3750C + 12500AB + 1250AC + 1250BC - 28750A^2 - 33750B^2 - 15000C^2$…… **(Eq. 8)**

Table 9: Response Table for Tretinoin

Run	Retention Time (min)	Tailing Factor	Peak Area (AU)
1	1.80	1.45	490000
2	1.30	1.40	520000
3	1.60	1.50	500000
4	1.22	1.45	560000
5	1.75	1.42	530000
6	1.28	1.38	570000
7	1.65	1.48	510000
8	1.25	1.44	565000
9	1.55	1.36	545000
10	1.35	1.41	550000
11	1.50	1.39	535000
12	1.30	1.43	548000
13	**1.22**	**1.30**	**560000**

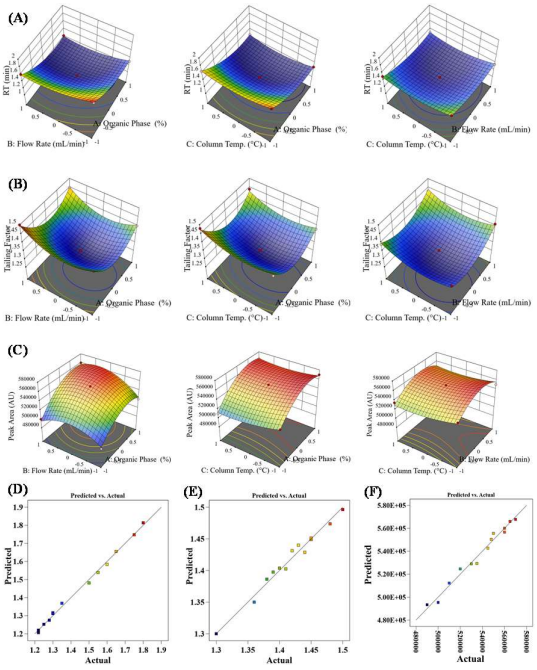

Fig. 1: Three-dimensional response surface plots of Tretinoin for the effect of independent variables on (A) Retention time, (B) Tailing factor, and (C) Peak area. Linear correlation plots between the actual and predicted values for (D) Retention time, (E) Tailing factor, and (F) Peak area.

Table 10: Response Table for Mequinol

Run	Retention Time (min)	Tailing Factor	Peak Area
1	2.90	1.50	820000
2	2.50	1.38	850000
3	2.70	1.46	800000
4	2.45	1.50	880000
5	2.85	1.38	830000
6	2.55	1.35	890000
7	2.75	1.44	820000
8	2.48	1.40	885000
9	2.65	1.34	850000
10	2.55	1.38	860000
11	2.70	1.36	840000
12	2.50	1.40	855000
13	**2.45**	**1.28**	**900000**

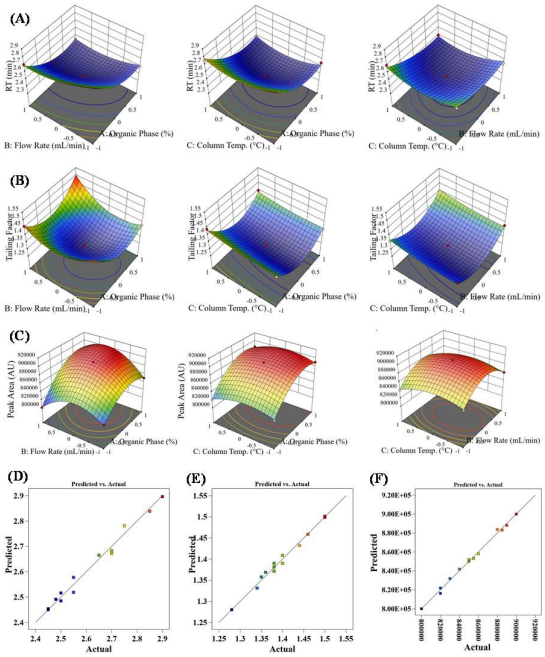

Fig. 2: Three-dimensional response surface plots of Mequinol for the effect of independent variables on (A) Retention time, (B) Tailing factor, and (C) Peak area. Linear correlation plots between the actual and predicted values for (D) Retention time, (E) Tailing factor, and (F) Peak area.

Linearity Assessment

The method was highly linear over the concentration range of 5 to 50 µg/mL. There was a strong linear relationship between peak area and concentration, indicating that the method was suitable for quantitative analysis. The linear regression equation to be:

y= mx+c…… … (Eq. 8)

Where, y is the peak area, and x is the concentration in µg/mL.

The correlation coefficient (R^2) was greater than 0.999 for both Tretinoin and Mequinol, indicating strong linearity.

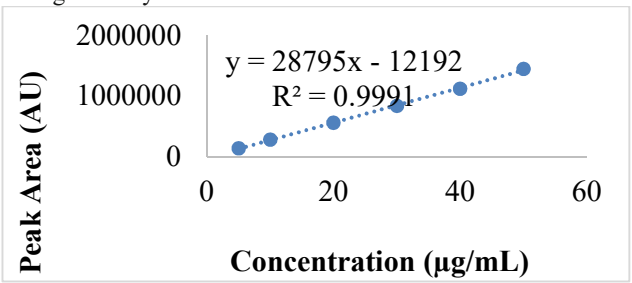

Fig. 3: Calibration plot of linearity for Tretinoin by RP-UPLC.

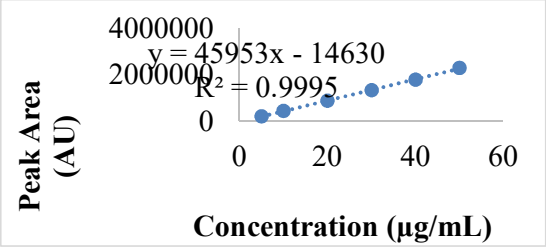

Fig. 4: Calibration plot of linearity for Mequinol by RP-UPLC.

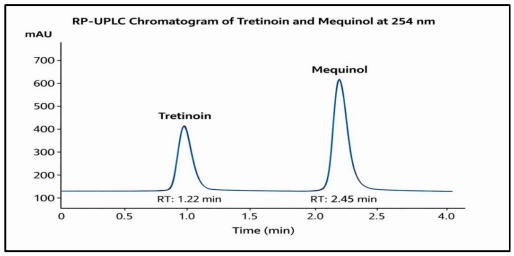

Fig. 5: RP-UPLC Chromatogram of Tretinoin and Mequinol at 254 nm

Optimization of Tretinoin–Mequinol Gel Formulation

A QbD-based Box–Behnken Design (BBD) was employed to optimize the Tretinoin–Mequinol gel formulation by investigating the effects of Carbopol concentration, propylene glycol concentration, and pH on drug release, viscosity, and spreadability. The experimental responses showed close agreement with the predicted values, confirming the suitability of the quadratic model for formulation optimization.

The optimized formulations exhibited drug release ranging from 65.4% to 92.6%, viscosity between 4200 and 9400 cP, and spreadability from 10.2 to 18.5 g·cm/sec. Statistical analysis demonstrated that the developed models were significant ($p < 0.05$), indicating that the selected formulation variables had a substantial influence on gel performance.

Among the studied factors, Carbopol concentration showed the greatest impact on all responses. Increasing Carbopol concentration significantly increased viscosity while reducing drug release and spreadability. In contrast, propylene glycol enhanced drug release and spreadability due to its solubilizing and penetration-enhancing properties. The effect of pH was comparatively less pronounced and mainly influenced gel consistency.

Response surface plots revealed that formulations containing lower Carbopol concentrations and higher levels of propylene glycol exhibited improved drug release and spreadability, whereas higher Carbopol levels produced more viscous gels. The predicted and experimental values showed excellent agreement, confirming the predictive capability of the developed models. High R^2 values (>0.98) and adequate precision values (>4) further demonstrated model robustness and reliability.

Optimization through desirability analysis identified the optimum formulation containing approximately 0.8–1.0% w/w Carbopol, 7–8% w/w propylene glycol, and a pH of 5.8–6.2. Under these conditions, the formulation exhibited drug release of 88–92%, viscosity of 6500–7500 cP, and spreadability of 17–18 g·cm/sec. These results indicate that the optimized gel possessed desirable physicochemical characteristics and was suitable for topical delivery of Tretinoin and Mequinol.

Table 11: Optimization of Tretinoin–Mequinol Gel Formulation

Run	Carbopol concentration (% w/w) (A)	Propylene glycol (% w/w) (B)	pH (Triethanolamine) (C)	Drug Release (%) (Y_1)	Viscosity (cP) (Y_2)	Spreadability (Y_3)
1	-1	-1	0	78.2	4200	15.8
2	+1	-1	0	65.4	8900	10.2
3	-1	+1	0	92.6	4800	18.5
4	+1	+1	0	80.1	9400	12.0
5	-1	0	-1	82.3	4500	16.2
6	+1	0	-1	70.2	9100	11.5
7	-1	0	+1	85.7	4700	17.1
8	+1	0	+1	73.5	9300	12.3
9	0	-1	-1	75.8	6000	14.2
10	0	+1	-1	88.9	6500	17.5
11	0	-1	+1	77.3	6200	14.8
12	0	+1	+1	90.4	6700	18.0
13	0	0	0	84.5	7100	16.5

Factors	Independent Variable	Level used, actual coded		
		Low (-1)	Medium (0)	High (+1)
A	Carbopol concentration (% w/w)	0.5	1.0	1.5
B	Propylene glycol (% w/w)	5.0	7.5	10.0

C	pH (Triethanolamine)	5.5	6.0	6.5

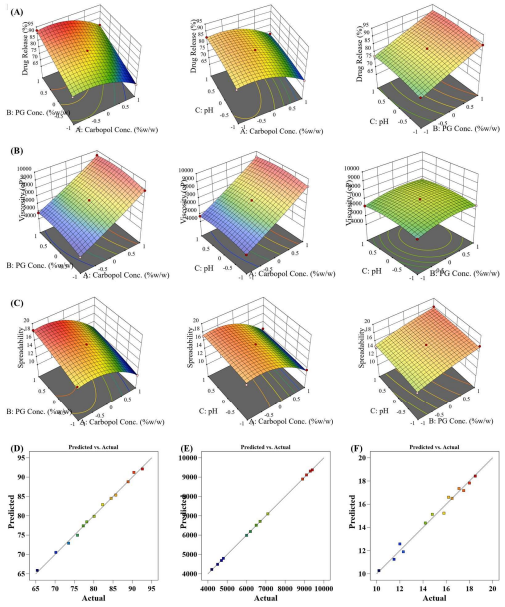

Fig. 6: Three-dimensional response surface plots for the effect of independent variables on (A) Drug release, (B) Viscosity, and (C) Spreadability. Linear correlation plots between the actual and predicted values for (D) Drug release, (E) **Viscosity, and** (F) **Spreadability**

Final Optimized Formulation Composition:

The optimized Tretinoin–Mequinol gel formulation for a 10 g batch consisted of Tretinoin (0.0025 g) and Mequinol (0.20 g) as active pharmaceutical ingredients, Carbopol 940 (0.09 g) as the gelling agent, and propylene glycol (0.75 g) as a solvent and penetration enhancer. To stabilize the formulation against microbial contamination, methyl paraben (0.01 g) was added as a preservative. Triethanolamine was also added in sufficient amount to bring the formulation pH to about 6.0, which helped the gel form correctly. Using purified water as a vehicle, the final volume was brought up to 10 g. This optimized composition was selected using a QbD-enabled Box–Behnken design to produce a balanced formulation with the desired drug release, viscosity, and spreadability properties for use on the skin.

Table 12: Optimized Formulation Composition (For 10 g Gel)

Sr. No.	**Ingredient**	**Function**	**Quantity**
1	Tretinoin	Retinoid (API)	0.0025 g (2.5 mg)
2	Mequinol	Depigmenting agent (API)	0.20 g (200 mg)
3	Carbopol 940	Gelling agent	0.09 g (90 mg)
4	Propylene Glycol	Solvent & penetration enhancer	0.75 g
5	Triethanolamine	pH adjuster	q.s. (to pH ~6.0)
6	Methyl Paraben	Preservative	0.01 g (10 mg)
7	Purified Water	Vehicle	q.s. to 10 g

***In Vitro* Drug Release Study:**

The optimized Tretinoin–Mequinol gel demonstrated a sustained and controlled drug release profile over 8 h. Tretinoin release increased progressively from 27.71% at 0.5 h to 95.78% at 8 h, while Mequinol release increased from 21.83% to 89.41% during the same period. Although Mequinol exhibited a slightly slower release than Tretinoin, both drugs showed a gradual and continuous release pattern. The enhanced release behavior may be attributed to the diffusion-controlled nature of the Carbopol gel matrix and the solubilizing effect of propylene glycol. The high cumulative drug release observed after 8 h indicates the suitability of the developed gel for prolonged topical delivery, which may improve therapeutic efficacy and reduce the frequency of application.

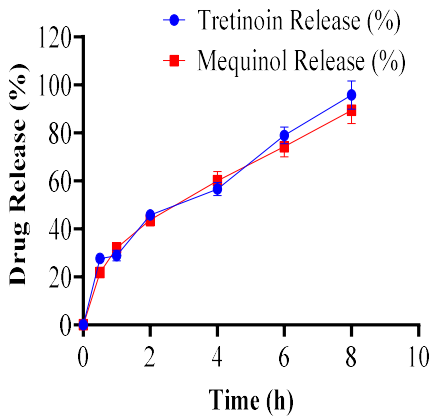

Fig. 7: Drug release across dialysis membrane for 8 h

Drug Release Kinetic Study

The release kinetics of Tretinoin and Mequinol from the optimized gel formulation were evaluated using different mathematical models (Table 19). Among the models tested, the Higuchi model showed the highest correlation coefficients (R^2 = 0.9873 for Tretinoin and 0.9910 for Mequinol), indicating that drug release was predominantly governed by diffusion through the gel matrix. The Korsmeyer–Peppas model further supported the release mechanism, with release exponent (*n*) values of 0.5264 and 0.743 for Tretinoin and Mequinol, respectively, suggesting anomalous (non-Fickian) transport involving both diffusion and polymer relaxation. These findings demonstrate that the developed gel provides a controlled and sustained drug release profile, making it suitable for prolonged topical delivery.

Table 13: Drug Release kinetic model study

Release kinetic models	Formulations	
	Tretinoin	**Mequinol**
Zero-order	0.9167	0.9377
First-order	0.9339	0.9542
Higuchi	**0.9873**	**0.9910**
Hixon-Crowell cube root	0.9645	0.9703
Korsmeyer-Peppas n = Release exponent	0.9695 (n = 0.5264)	0.9601 (n = 0.743)

CONCLUSION:

A Quality-by-Design-based approach was successfully employed for the development and optimization of a topical combination gel containing Tretinoin and Mequinol. The RP-UPLC method developed for simultaneous estimation of both drugs demonstrated excellent linearity, sensitivity, and reliability, making it suitable for routine quality control and formulation analysis. The Box–Behnken Design effectively identified the critical formulation variables influencing drug release, viscosity, and spreadability, enabling the selection of an optimized formulation with desirable physicochemical properties. The optimized gel formulation exhibited satisfactory viscosity, good spreadability, and sustained release characteristics, achieving cumulative drug releases of 95.78% for Tretinoin and 89.41% for Mequinol over 8 h. Drug release kinetics were best described by the Higuchi model, indicating diffusion-controlled release from the Carbopol gel matrix. The formulation also maintained its quality attributes during accelerated stability studies, confirming its physical and chemical stability. The findings demonstrate that the developed Tretinoin–Mequinol gel is a stable, effective, and patient-friendly topical delivery system. The integration of QbD principles with statistical optimization provided a robust formulation development strategy and ensured consistent product performance. This optimized formulation has significant potential for the sustained topical treatment of hyperpigmentation disorders and may serve as a promising platform for future dermatological applications.

CONFLICT OF INTEREST:

The authors declare that there are no conflicts of interest.

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