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Design of Experiments-Assisted Development and Validation of an HPTLC Method for Simultaneous Quantification of Silodosin and Tadalafil in Pharmaceutical Formulation

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Article

Keywords: Silodosin, Tadalafil, HPTLC, Design of Experiments, Pharmaceutical analysis

Posted Date: April 23rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9343659/v1

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Additional Declarations: No competing interests reported.

Abstract

A robust and efficient high-performance thin-layer chromatographic (HPTLC) method was developed for the simultaneous quantification of silodosin and tadalafil in pharmaceutical formulations. Method optimization was performed using a Design of Experiments (DoE) approach based on central composite design, enabling systematic evaluation of critical chromatographic variables including chamber saturation time, migration distance, and ammonia concentration.

Chromatographic separation was achieved on silica gel 60 F254 plates using a mobile phase of ethyl acetate, ammonia, and methanol (9:1:0.3, v/v/v), with densitometric detection at 260 nm. The method exhibited excellent linearity across the studied concentration ranges, with strong correlation coefficients. Accuracy studies demonstrated recoveries within acceptable limits, while precision assessment indicated low variability, confirming method reproducibility. Sensitivity evaluation showed that the method is capable of detecting and quantifying analytes at low levels.

The DoE strategy enabled identification of an optimal design space, enhancing method robustness and reducing experimental variability compared to conventional optimization approaches. The validated method is simple, cost-effective, and suitable for routine quality control analysis of combined pharmaceutical formulations.

INTRODUCTION

Benign prostatic hyperplasia (BPH) is a chronic, progressive urological condition characterized by enlargement of the prostate gland and associated lower urinary tract symptoms, including urinary frequency, urgency, and reduced flow rate [1, 2]. The multifactorial nature of BPH has led to the use of combination pharmacotherapy targeting different physiological pathways to achieve improved therapeutic outcomes.

Silodosin is a highly selective α1A-adrenoceptor antagonist that exerts its pharmacological effect by inhibiting adrenergic stimulation of smooth muscle in the prostate and bladder neck, thereby reducing urethral resistance and improving urinary flow [3]. In contrast, tadalafil is a phosphodiesterase type-5 (PDE-5) inhibitor that enhances intracellular cyclic guanosine monophosphate (cGMP) levels, leading to smooth muscle relaxation and increased blood perfusion in the lower urinary tract [4, 5]. The complementary mechanisms of these two drugs make their combination particularly effective in managing BPH symptoms.

From an analytical perspective, the structural features of silodosin and tadalafil (Fig. 1), including aromatic rings, heterocyclic systems, and polar functional groups, significantly influence their chromatographic behavior. These characteristics affect adsorption, migration, and separation efficiency on silica-based stationary phases, necessitating careful selection and optimization of chromatographic conditions [6].

High-performance thin-layer chromatography (HPTLC) has emerged as a versatile analytical technique in pharmaceutical analysis due to its advantages such as low solvent consumption, rapid analysis, minimal sample preparation, and the ability to analyze multiple samples simultaneously [7, 8]. Despite these benefits, the performance of HPTLC methods is highly dependent on several experimental variables, including mobile phase composition, chamber saturation conditions, and solvent migration distance.

Conventional method development approaches often rely on one-factor-at-a-time experimentation, which may fail to capture interactions between variables and can lead to suboptimal method performance. In contrast, Design of Experiments (DoE) provides a systematic and multivariate framework for method optimization. By simultaneously evaluating multiple factors and their interactions, DoE enhances method understanding and robustness. Central composite design (CCD), a widely used response surface methodology, enables efficient exploration of experimental space and identification of optimal operating conditions with reduced experimental effort [9–12].

Analytical method validation is essential to ensure the reliability, reproducibility, and suitability of the developed method for its intended purpose. The International Council for Harmonisation (ICH) Q2(R2) guideline provides a comprehensive framework for validation, encompassing parameters such as specificity, linearity, accuracy, precision, detection limit, quantification limit, and robustness [13]. Compliance with these guidelines ensures that the method produces consistent and scientifically sound results suitable for regulatory and quality control applications.

Although several analytical methods have been reported for individual estimation of silodosin or tadalafil, there is limited literature addressing their simultaneous quantification using a statistically optimized HPTLC approach. Therefore, the present study aims to develop a DoE-assisted HPTLC method for simultaneous estimation of silodosin and tadalafil in pharmaceutical formulations and to validate the method in accordance with ICH Q2(R2) [14] guidelines.

MATERIALS AND METHODOLOGY

Chemicals and reagents

Reference standards of silodosin and tadalafil (purity ≥ 99%) were obtained from authenticated pharmaceutical sources and used without further purification. Methanol, ethyl acetate, and ammonia solution were of analytical grade and procured from reliable commercial suppliers. Pre-coated silica gel 60 F254 aluminum-backed plates (20 × 10 cm) were used as the stationary phase. All solvents were freshly prepared and filtered prior to use.

Instrumentation and chromatographic conditions

Chromatographic analysis was carried out using a CAMAG high-performance thin-layer chromatography (HPTLC) system comprising a Linomat 5 sample applicator equipped with a Hamilton syringe, a twin-

trough glass development chamber, and a TLC Scanner 4 operated through winCATS software.

Sample application was performed as bands of uniform width under a controlled nitrogen stream to ensure reproducibility. Chromatographic separation was achieved on silica gel 60 F254 plates using a mobile phase consisting of ethyl acetate, ammonia, and methanol in an optimized ratio. Prior to development, the chamber was allowed to equilibrate with mobile phase vapor for an appropriate duration to ensure consistent saturation conditions. Plate development was conducted up to a defined migration distance under ambient laboratory conditions. After development, plates were dried and subjected to densitometric scanning at 260 nm in absorbance mode.

Preparation of standard solutions

Primary stock solutions of silodosin and tadalafil were prepared separately by accurately weighing and dissolving each drug in methanol to obtain a concentration of 1000 µg/mL. Working standard solutions were subsequently prepared by appropriate dilution of the stock solutions with methanol to achieve concentrations within the desired analytical range.

Preparation of sample solution

Pharmaceutical formulation containing both analytes was accurately weighed and finely powdered. A quantity equivalent to the labeled drug content was transferred into a volumetric flask and extracted with methanol using sonication for 15 minutes to ensure complete dissolution. The extract was filtered to remove insoluble excipients, and the filtrate was suitably diluted with methanol to obtain concentrations within the working range. The prepared sample solution was applied under the same chromatographic conditions as the standard solutions.

Experimental design for method optimization

A systematic optimization strategy based on Design of Experiments (DoE) was employed to evaluate the influence of critical chromatographic parameters on method performance. A central composite design was selected to study the combined effects of chamber saturation time, solvent migration distance, and ammonia concentration in the mobile phase.

Each factor was investigated at multiple levels to capture both linear and quadratic effects. The experimental data were statistically analyzed using analysis of variance to determine the significance of individual variables and their interactions. Response surface methodology was applied to identify optimal operating conditions and to establish a robust analytical design space.

Method validation

The developed HPTLC method was validated in accordance with International Council for Harmonisation (ICH) Q2(R2) [14] guidelines to ensure its suitability for quantitative analysis of silodosin and tadalafil.

Working Range

Working Range was established by applying a series of standard solutions at different concentration levels covering the expected analytical range. Each concentration was applied in triplicate to ensure consistency. The chromatograms were developed under optimized conditions, and peak areas were recorded. Calibration curves were constructed by plotting peak area versus corresponding concentration, and regression analysis was performed using the least squares method. The linearity of the method was evaluated based on the correlation coefficient and residual distribution.

Accuracy (recovery studies)

Accuracy was evaluated using the standard addition method to account for potential matrix effects. Pre-analyzed sample solutions were spiked with known quantities of silodosin and tadalafil at three concentration levels (typically 80%, 100%, and 120% of the nominal concentration). Each level was analyzed in triplicate. The mixtures were processed under the same chromatographic conditions, and the percentage recovery was calculated by comparing the measured concentration with the added amount.

Precision

Precision of the method was assessed at two levels:

- Repeatability (intra-day precision): Multiple replicate analyses of the same concentration were performed within a single day under identical conditions.
- Intermediate precision (inter-day precision): The same procedure was repeated on different days to evaluate variability over time.

For both cases, peak areas were recorded and expressed as percentage relative standard deviation (%RSD) to assess the degree of variation.

Sensitivity

The sensitivity of the method was determined by calculating the limit of detection (LOD) and limit of quantification (LOQ) based on the standard deviation of the response and the slope of the calibration curve. The standard deviation was obtained from replicate measurements of low concentration samples. The calculations were performed using the following relationships:

$LOD = 3.3 \times (\sigma/S)$

$LOQ = 10 \times (\sigma/S)$

where σ represents the standard deviation of the response and S denotes the slope of the calibration curve.

Robustness

Robustness was evaluated by deliberately introducing small variations in key chromatographic parameters, such as slight changes in mobile phase composition, chamber saturation time, and detection wavelength. The effect of these variations on peak area and retention behavior was examined. The method was considered robust if these changes did not produce significant variation in analytical response.

Specificity

Specificity was assessed by analyzing blank, standard, and sample solutions to ensure that the analytes were accurately identified and quantified without interference from excipients or other components. The separation was considered specific when well-resolved peaks corresponding to silodosin and tadalafil were obtained with no co-eluting impurities.

RESULTS

Chromatographic separation and system performance

The optimized chromatographic conditions produced well-defined, compact, and symmetrical peaks for both analytes. The densitogram (Fig. 2) demonstrated clear separation of silodosin and tadalafil without interference from excipients or background noise. The retention factor (Rf) values were found to be consistent across multiple runs, confirming system suitability and reproducibility of the developed method. Under optimized conditions, silodosin and tadalafil exhibited Rf values of approximately 0.254 and 0.697, respectively, indicating effective resolution between the two compounds.

Optimization using Design of Experiments (DoE)

A central composite design (CCD) was employed to systematically evaluate the effect of critical chromatographic variables, including chamber saturation time, solvent front migration distance, and ammonia concentration in the mobile phase. The experimental design matrix and observed responses are presented in Table 1, while statistical evaluation using ANOVA is summarized in Table 2. The results indicated that chamber saturation time significantly influenced retention behavior, whereas ammonia concentration played a crucial role in improving peak separation. Interaction effects between variables were evident and further visualized through contour plots and response surface plots (Fig. 4 **and** Fig. 5). These findings confirm the suitability of the quadratic model and enabled identification of an optimized and robust design space.

Table 1
Central Composite Design (CCD) experimental matrix and observed responses for HPTLC optimization of silodosin and tadalafil.

Run	Saturation Time (min)	Solvent Front (mm)	Ammonia (mL)	Rf (Silodosin)	Peak Height (Silodosin)	Rf (Tadalafil)	Peak Height (Tadalafil)
1	21	79	0.2	0.21	0.23	0.69	0.24
2	21	81	0.2	0.38	0.47	0.67	0.46
3	21	79	0.4	0.40	0.34	0.75	0.33
4	20	80	0.3	0.20	0.31	0.67	0.34
5	20	81	0.3	0.20	0.30	0.64	0.46
6	20	80	0.3	0.27	0.37	0.69	0.43
7	19	79	0.2	0.19	0.25	0.66	0.36
8	19	79	0.4	0.17	0.26	0.61	0.22
9	19	81	0.4	0.24	0.17	0.76	0.17
10	21	80	0.3	0.32	0.41	0.63	0.44
11	20	79	0.3	0.28	0.43	0.63	0.36
12	19	80	0.3	0.18	0.29	0.65	0.32
13	21	81	0.4	0.37	0.48	0.73	0.42
14	20	80	0.3	0.25	0.22	0.69	0.28
15	20	80	0.4	0.32	0.25	0.62	0.21
16	19	81	0.2	0.18	0.13	0.66	0.31
17	20	80	0.2	0.31	0.42	0.72	0.42

The table presents the experimental runs generated using CCD showing the effect of chamber saturation time, solvent front migration distance, and ammonia concentration on retention factor (Rf) and peak height responses of silodosin and tadalafil.

Table 2
Analysis of variance (ANOVA) summary for the DoE models applied to silodosin and tadalafil responses.

Parameter / Factor	Response	Drug	Model Type	F-value	p-value	Significance
Saturation Time (A)	Rf	Silodosin	Linear	Significant	< 0.05	Significant
Solvent Front (B)	Rf	Silodosin	Linear	Low effect	> 0.05	Not Significant
Ammonia in Mobile Phase (C)	Rf	Silodosin	Linear	Minor effect	> 0.05	Not Significant
Lack of Fit	Rf	Silodosin	Linear	~ 2.17	0.3580	Not Significant
Saturation Time (A)	Height	Silodosin	2FI	Moderate	< 0.05	Significant
Solvent Front (B)	Height	Silodosin	2FI	Minor	> 0.05	Not Significant
Ammonia (C)	Height	Silodosin	2FI	Minor	> 0.05	Not Significant
Saturation Time (A)	Rf	Tadalafil	Linear	Moderate	< 0.05	Significant
Solvent Front (B)	Rf	Tadalafil	Linear	Moderate	< 0.05	Significant
Ammonia (C)	Rf	Tadalafil	Linear	Small effect	> 0.05	Not Significant
Lack of Fit	Rf	Tadalafil	Linear	Acceptable	> 0.05	Not Significant
Saturation Time (A)	Height	Tadalafil	Linear	Moderate	< 0.05	Significant
Solvent Front (B)	Height	Tadalafil	Linear	Moderate	< 0.05	Significant
Ammonia (C)	Height	Tadalafil	Linear	Negative effect	> 0.05	Not Significant

Statistical evaluation of the significance of chromatographic variables including saturation time, solvent front migration distance, and ammonia concentration on retention factor and peak height responses.

Optimized chromatographic conditions

The optimized chromatographic conditions consisted of a mobile phase composed of ethyl acetate, ammonia, and methanol in the ratio of 9:1:0.3 (v/v/v). Under these conditions, silodosin and tadalafil

showed Rf values of 0.254 and 0.697, respectively, while the mixture exhibited Rf values of 0.224 for silodosin and 0.704 for tadalafil. The separation was found to be clear and reproducible, indicating suitability of the method for simultaneous estimation (Table 3).

Table 3
Optimized chromatographic conditions and observed Rf values for silodosin and tadalafil.

Parameter	Condition / Observation
Mobile Phase	Ethyl acetate: Ammonia: Methanol (9:1:0.3, v/v/v)
Rf (Silodosin)	0.254
Rf (Tadalafil)	0.697
Rf (Mixture)	Silodosin = 0.224; Tadalafil = 0.704
Observation	All drugs were well resolved and clearly separated

Final optimized mobile phase composition and the corresponding retention factors demonstrating effective separation of both analytes under HPTLC conditions.

Working range and linearity

The method demonstrated a linear response over the concentration range of 1200–7200 ng/band for silodosin and 750–4500 ng/band for tadalafil. Calibration curves constructed by plotting peak area versus concentration showed a strong linear relationship for both analytes (Fig. 3). Regression analysis yielded correlation coefficients (R^2) of 0.9872 for silodosin and 0.9777 for tadalafil, indicating good linearity. The regression equations further confirmed proportionality between concentration and analytical response (Table 4).

Table 4
Linearity data and regression parameters for silodosin and tadalafil.

Silodosin		**Tadalafil**	
Concentration (ng/spot)	**Peak Area**	**Concentration (ng/spot)**	**Peak Area**
1200	0.00686	750	0.01545
2400	0.00801	1500	0.01793
3600	0.00862	2250	0.01981
4800	0.00909	3000	0.02116
6000	0.00999	3750	0.02253
7200	0.01078	4500	0.02363
Regression Equation	y =6E-07x + 0.0063	**Regression Equation**	Y=2E-06x + 0.0145
R^2	0.9872	**R^2**	0.9777
Slope	0.0000006	**Slope**	0.000002
Intercept	0.0063	**Intercept**	0.0145
Linearity	1200–7200 ng	**Linearity**	750–4500 ng

Calibration data showing the relationship between concentration and peak area along with regression equation, slope, intercept, and correlation coefficient for both analytes.

Accuracy (recovery studies)

Accuracy was evaluated using the standard addition method at three levels (80%, 100%, and 120%). The percentage recovery values for silodosin ranged from 100.13% to 101.17%, while for tadalafil they ranged from 99.99% to 100.37%. The mean recovery values were close to 100%, with low standard deviation and %RSD values, confirming the accuracy of the method and absence of matrix interference (Table 5).

Table 5
Precision study results for silodosin and tadalafil

Drug	Precision Type	Concentration (ng/band)	Mean Peak Area ± SD	%RSD
Silodosin	Repeatability	1200	0.006845 ± 0.0000187	0.273
	Intraday	1200	0.00685 ± 0.0000265	0.938
		3600	0.00863 ± 0.0000270	0.306
		6000	0.00991 ± 0.0000643	0.648
	Interday	1200	0.01544 ± 0.0000153	0.099
		3600	0.01981 ± 0.0000115	0.058
		6000	0.02252 ± 0.00000577	0.025
Tadalafil	Repeatability	750	0.01544 ± 0.00000983	0.078
	Intraday	750	0.01544 ± 0.0000153	0.171
		2250	0.01981 ± 0.0000115	0.219
		3750	0.02252 ± 0.00000577	0.067
	Interday	750	0.01544 ± 0.00000577	0.171
		2250	0.01983 ± 0.0000436	0.219
		3750	0.02255 ± 0.0000153	0.067

Evaluation of repeatability, intra-day, and inter-day precision expressed as mean peak area ± standard deviation and percentage relative standard deviation (%RSD).

Precision

Precision of the method was assessed in terms of repeatability (intra-day) and intermediate precision (inter-day). The %RSD values for silodosin ranged from 0.025% to 0.938%, while for tadalafil they ranged from 0.067% to 0.219%. All values were well within the acceptable limit of less than 2%, demonstrating excellent repeatability and reproducibility of the method under both intra-day and inter-day conditions (Table 6).

Table 6
Accuracy (recovery) study of silodosin and tadalafil using the standard addition method.

Drug	Level (% Recovery)	Sample Amount (ng/spot)	Spiked Amount (ng/spot)	Mean Total Amount Recovered (ng/spot)	Mean % Recovery	SD	% RSD
Silodosin	80%	200	300	500.83	100.16	1.5275	1.52
	100%	200	400	600.83	100.13	1.6821	1.67
	120%	200	500	708.33	101.17	1.3375	1.32
Tadalafil	80%	200	300	501.33	100.26	1.1015	1.09
	100%	200	400	600.00	99.99	1.2583	1.25
	120%	200	500	702.67	100.37	0.6768	0.67

Mean percentage recovery, standard deviation, and %RSD values obtained at 80%, 100%, and 120% spiking levels.

Sensitivity (LOD and LOQ)

The sensitivity of the method was evaluated by determining the limit of detection (LOD) and limit of quantification (LOQ). The LOD values were found to be 7696.18 ng for silodosin and 4999.44 ng for tadalafil, while the LOQ values were 23321.76 ng and 16498.15 ng, respectively. These results indicate that the method is sufficiently sensitive for detection and quantification of both analytes at low concentration levels.

Robustness

Robustness was evaluated by introducing small deliberate variations in chromatographic conditions, including changes in mobile phase composition and chamber saturation time. The results indicated that such variations did not produce any significant effect on Rf values or peak characteristics. The method maintained consistent performance with clear separation under varied conditions, confirming its robustness and reliability for routine analysis (Table 7).

Table 7
Robustness study of the developed HPTLC method.

Parameter Changed	Condition Applied	Observation (Silodosin)	Observation (Tadalafil)	Remark
Mobile phase composition	Ethyl acetate: Ammonia: Methanol (9:1:0.5 v/v/v)	Rf = 0.240	Rf = 0.707	No significant change
Chamber saturation time	20 min	Proper peak observed	Proper peak observed	Clear separation

Effect of deliberate variations in chromatographic parameters including mobile phase composition and chamber saturation time on retention behavior of silodosin and tadalafil.

Specificity

Specificity of the method was confirmed by analyzing blank, standard, and sample solutions. The densitogram (Fig. 2) showed well-resolved peaks corresponding to silodosin and tadalafil without any interference from excipients or other components. This demonstrates that the method is selective and suitable for simultaneous estimation of both analytes in pharmaceutical formulations.

DISCUSSION

The present study demonstrates the systematic development of a robust HPTLC method for the simultaneous estimation of silodosin and tadalafil using a Design of Experiments (DoE)-assisted approach. Initial wavelength selection based on UV spectral analysis ensured adequate sensitivity and selectivity, enabling reliable densitometric quantification of both analytes. Preliminary chromatographic investigations revealed the limitations of conventional solvent systems such as toluene–chloroform and acetonitrile–methanol, which failed to achieve satisfactory resolution. In contrast, ethyl acetate-based systems provided superior selectivity, and the optimized mobile phase comprising ethyl acetate, ammonia, and methanol resulted in well-resolved peaks with consistent migration behavior. This improvement can be attributed to the optimized polarity and the basic environment introduced by ammonia, which enhances analyte–stationary phase interactions. The application of central composite design enabled efficient multivariate optimization of critical chromatographic parameters, including chamber saturation time, solvent front migration distance, and ammonia concentration. Statistical analysis identified chamber saturation time as a dominant factor influencing retention behavior, particularly for silodosin, while ammonia concentration significantly affected tadalafil migration. The observed interaction effects highlight the importance of multivariate approaches over conventional univariate optimization. Response surface and perturbation analyses further confirmed the non-linear relationships between variables and their combined impact on chromatographic performance. Notably, the relatively minor influence of solvent front distance compared to saturation time emphasizes the critical role of chamber equilibrium conditions in HPTLC separations.

The optimized method exhibited stable Rf values and consistent peak responses, indicating high robustness. Validation studies further confirmed method reliability. The established linearity ranges demonstrated strong correlation coefficients, indicating proportional analytical response across the studied concentrations. Precision results, with %RSD values well below 2%, confirm excellent repeatability and intermediate precision. Accuracy studies yielded recovery values within 98–102%, demonstrating the absence of matrix interference and suitability for quantitative analysis. Sensitivity assessment indicated adequate detection capability, although the comparatively higher LOQ values suggest that the method is more appropriate for routine quality control rather than trace-level analysis. Robustness evaluation showed minimal impact of deliberate variations in experimental conditions, confirming method stability. Additionally, the absence of interfering peaks establishes the specificity of the method. Overall, the integration of DoE significantly enhanced method understanding, reduced experimental effort, and enabled the development of a statistically optimized and reliable analytical procedure.

CONCLUSION

A robust, efficient, and reproducible HPTLC method for the simultaneous estimation of silodosin and tadalafil in pharmaceutical formulations was successfully developed and validated. The application of a DoE-based optimization strategy enabled systematic evaluation of critical chromatographic parameters, resulting in improved separation efficiency and enhanced method reliability. The method demonstrated satisfactory performance in terms of linearity, accuracy, precision, specificity, robustness, and sensitivity in accordance with ICH Q2(R2) guidelines.

The optimized chromatographic conditions provided clear and well-resolved peaks with consistent retention behavior. The method is simple, cost-effective, and suitable for routine quality control analysis of combined dosage forms. Furthermore, the implementation of DoE minimized experimental trials while improving process understanding, making the approach both efficient and scientifically rigorous. The developed method can be effectively applied in pharmaceutical industries for the analysis of formulations containing silodosin and tadalafil.

NOVELTY STATEMENT

This study presents a novel DoE-assisted strategy for the systematic development and optimization of an HPTLC method for the simultaneous estimation of silodosin and tadalafil in pharmaceutical formulations. Unlike conventional trial-and-error approaches, the use of central composite design enabled comprehensive evaluation of critical chromatographic variables and their interactions, leading to the establishment of a statistically robust design space. To the best of our knowledge, limited studies have reported the simultaneous estimation of silodosin and tadalafil using HPTLC, and none have incorporated multivariate optimization techniques for method development. The proposed approach offers improved efficiency, reduced solvent consumption, and enhanced analytical performance compared to traditional methods. Moreover, validation in accordance with ICH Q2(R2) guidelines

ensures the reliability and applicability of the method for routine quality control. The integration of DoE with HPTLC not only reduces experimental workload but also provides deeper insight into variable interactions, thereby enhancing both scientific rigor and practical applicability.

Declarations

ACKNOWLEDGEMENTS

We express our gratitude to Parul University, Parul Institute of Pharmacy and Research, for offering laboratory facilities, encouragement, and continuous support throughout the research process. Finally, we appreciate all individuals who contributed to this work for their cooperation and valuable assistance.

Author's contributions

Pooja Chafle and Prexa Tandel have performed the experimental work and drafting. Hiralben Mehta and G. S. Chakraborthy have supervised the study and critically revised the manuscript. All authors reviewed and approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

Data Availability

All data generated or analyzed during this study are included in this published article.

Ethics declaration and consent to participate

Not applicable.

Consent for publication

Not applicable.

Funding

Open access funding provided by Parul University. None received.

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Figures

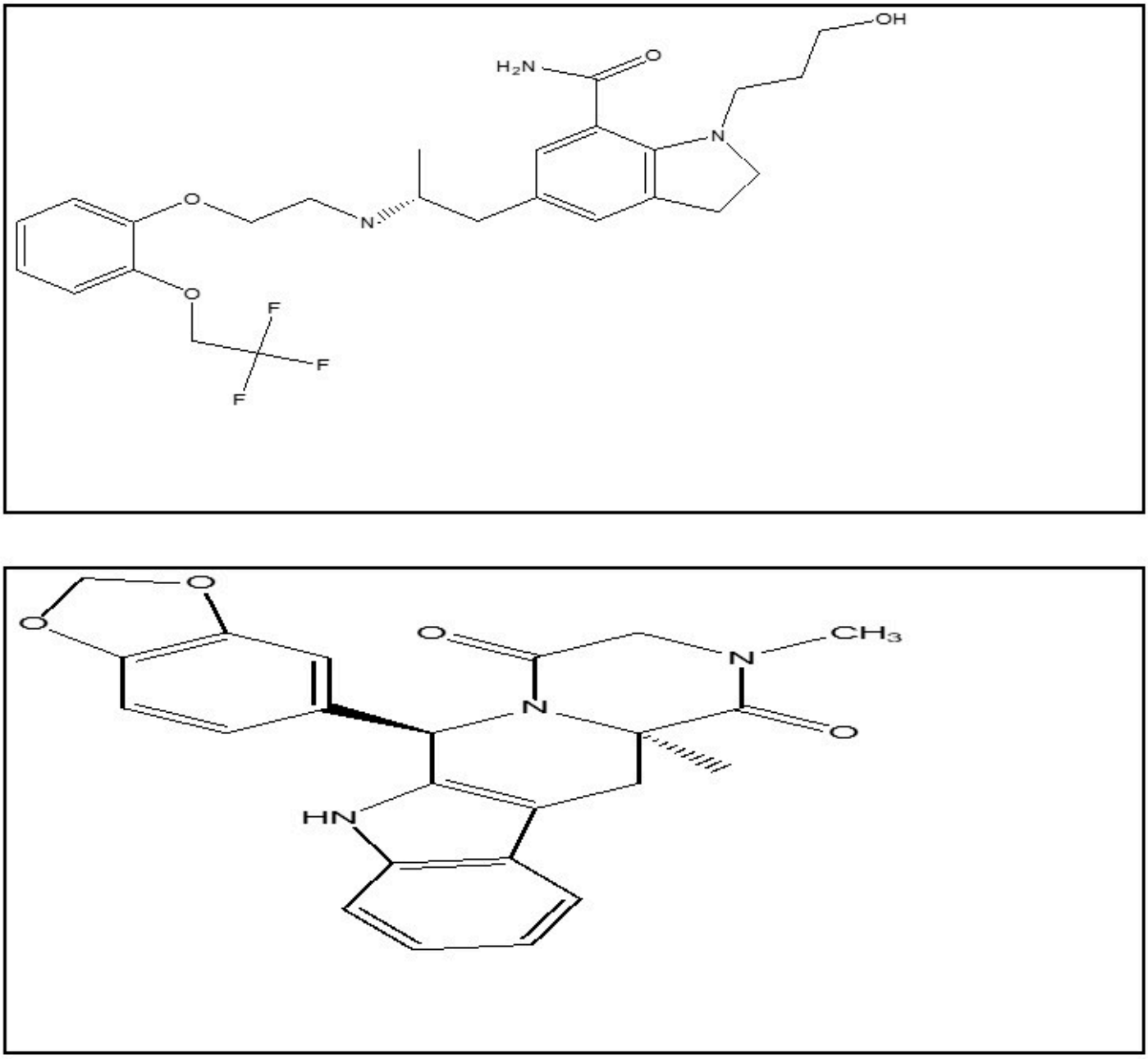


Figure 1

Chemical structures of silodosin and tadalafil.

Chemical structures highlighting the functional groups that influence chromatographic interaction and migration behavior during HPTLC separation.

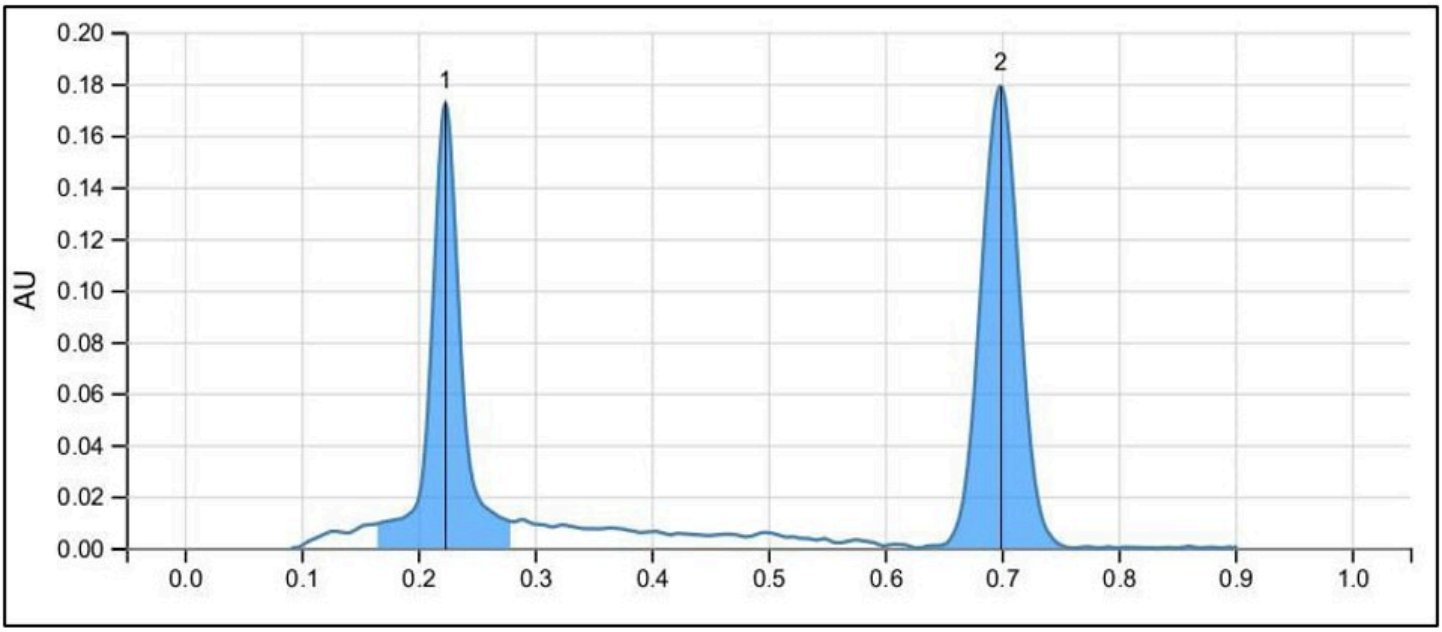


Figure 2

HPTLC densitogram of silodosin and tadalafil under optimized chromatographic conditions.

Densitogram showing well-resolved peaks corresponding to silodosin and tadalafil without interference, confirming method specificity.

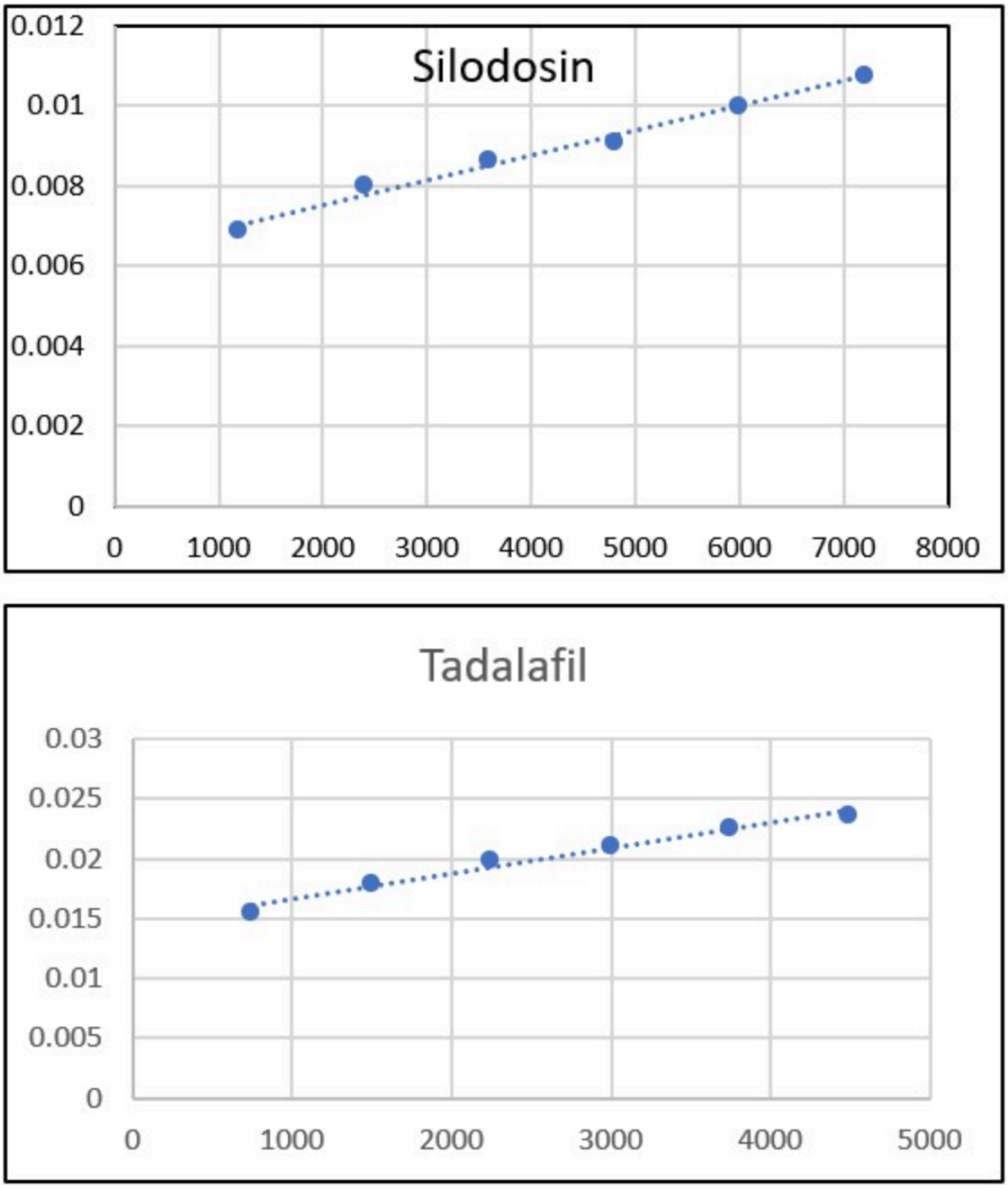


Figure 3

Calibration curves of silodosin and tadalafil.

Linear relationship between peak area and concentration demonstrating method linearity across the studied working ranges.

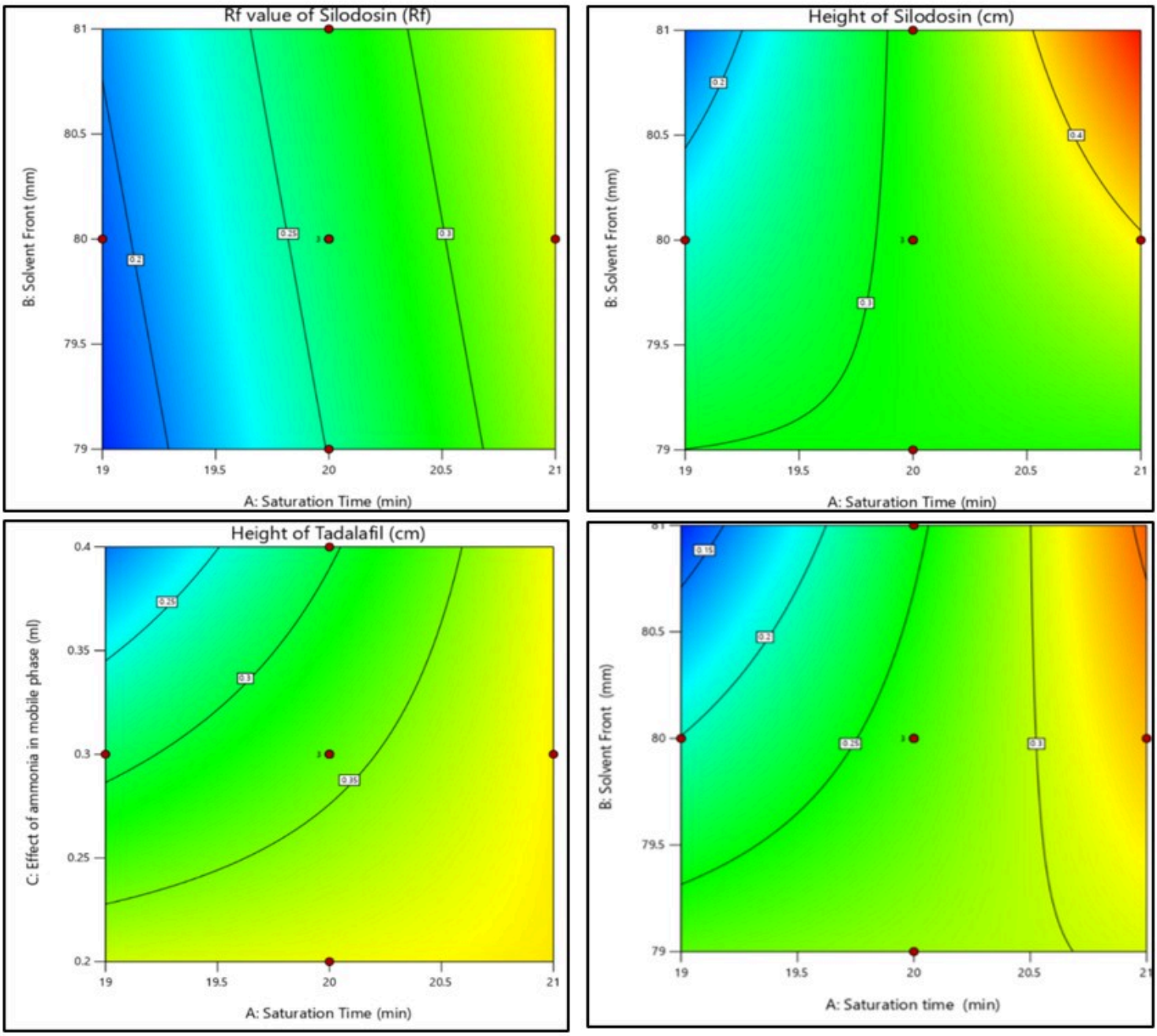


Figure 4

Contour plots showing interaction effects of chromatographic variables.

Two-dimensional contour plots illustrating the influence of chamber saturation time, solvent front migration distance, and ammonia concentration on chromatographic separation.

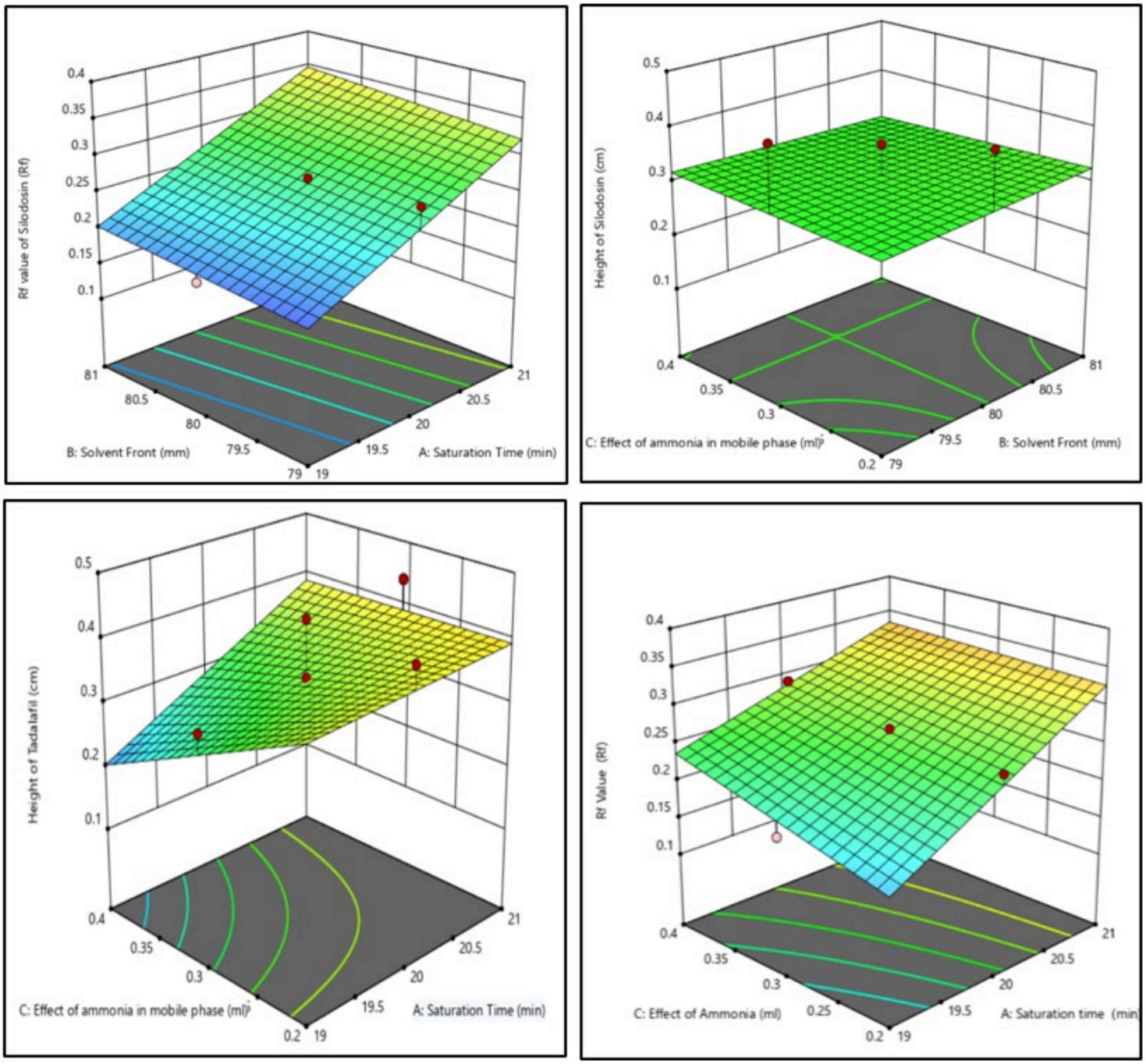


Figure 5

Three-dimensional response surface plots of chromatographic responses.

Response surface plots depicting the combined effect of critical experimental factors on retention behavior and peak response of the analytes.

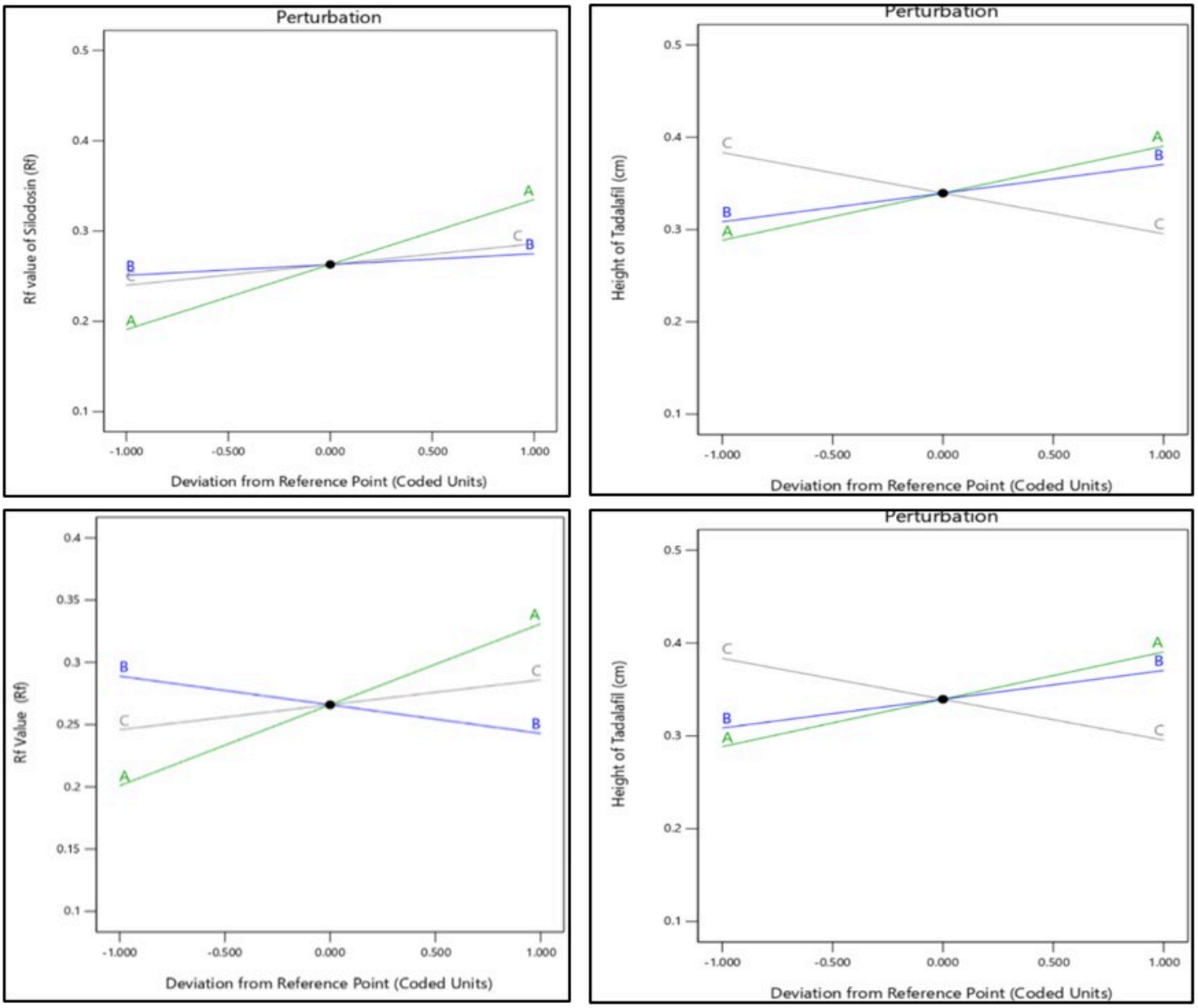


Figure 6

Perturbation plot illustrating the influence of chromatographic variables on analytical response.

Perturbation analysis demonstrating the relative sensitivity of the analytical response to changes in chamber saturation time, migration distance, and ammonia concentration.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- floatimage1.png