ORIGINAL ARTICLE

Quality by Design-based Stability-indicating Reverse Phase High Performance Liquid Chromatography Method Development and Validation for Simultaneous Estimation of Tamsulosin Hydrochloride and Tadalafil in Pharmaceutical Dosage Forms

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Abstract

A stability-indicating reverse phase high performance liquid chromatography (RP-HPLC) method based on the analytical quality by design (AQbD) approach was developed and validated for the simultaneous estimation of Tamsulosin (TAM) hydrochloride (HCl) and Tadalafil (TDL) in pharmaceutical formulations. An analytical target profile was developed that specified the method performance requirements, and critical method parameters were identified based on risk assessment, and optimized using a systematic experimental design. Chromatographic separation was available on the C18 column with triethylamine buffer and methanol as the mobile phase and detection was performed at 243 nm using ultraviolet. The method was validated as per the International Conference on Harmonisation Q2(R1) guidelines for linearity, precision, accuracy, specificity, robustness, and sensitivity. The method showed a good linearity within the studied concentration ranges with a correlation coefficient of more than 0.999 for both analytes. Precision studies provided ≤2% relative standard deviation values, indicating good repeatability, and intermediate precision and accuracy studies gave the recovery values between >98% and 102%.The limits of detection were found to be 0.4 µg/mL for TAM HCl and 1.3 µg/mL for TDL. Robustness evaluation revealed that slight changes of chromatographic conditions did not influence the performance of the method to a great extent. Forced degradation studies under acidic, alkaline, oxidative, thermal, and photolytic conditions ensured good separation of degradation products from the analyte peaks, proving the stability-indicating ability of the method. The developed AQbD-driven RP-HPLC method is precise, accurate, robust, and reliable, and thus can be used for routine quality control and stability studies of TAM HCl and TDL in both dosage combinations.

Key words: Analytical quality by design, method validation, reverse phase high performance liquid chromatography, tadalafil, tamsulosin

INTRODUCTION

Analytical method reliability is important for the quality, safety, and performance of pharmaceutical products. Conventional approaches to method development are often empirical in nature and may provide little insight into method variability and robustness.[1] To overcome these limitations, the quality by

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design (QbD) idea has been implemented as a systematic, science-driven approach giving emphasis on identification of critical factors, process understanding, and identification of design space with predefined quality objectives.[2] When applied to analytical method development, this approach is termed Analytical QbD (AQbD), which is a risk assessment, experimental design, lifecycle management approach to improve the robustness of analytical methods and improve regulatory compliance.[3]

Tamsulosin (TAM) hydrochloride (HCl) is a selective a1A-adrenergic receptor antagonist drug used widely in the treatment of benign prostatic hyperplasia, whereas Tadalafil (TDL) is a selective phosphodiesterase-5 inhibitor indicated for erectile dysfunction and pulmonary arterial hypertension.[4] The growing therapeutic importance of their combined application has resulted in the production of fixed-dose combination preparations, including the requirement for accurate and reliable analytical methods for their simultaneous estimation.[5]

Although several analytical methods for the determination of TAM and TDL have been reported, many of them do not contain systematic optimization, robustness evaluation, and stability – indicating assessment. Furthermore, there have been few studies in which AQbD principles have been applied in the development of chromatographic methods for such combination products. Therefore, the aim of the present study was to achieve the development of a stability-indicating reverse phase high performance liquid chromatography (RP-HPLC) method using the AQbD framework and validate it following the International Conference on Harmonisation (ICH) guidelines, with the help of creating a robust and scientifically justified tool for routine study purposes in terms of quality control and stability of combined pharmaceutical formulation.[6-12]

MATERIALS AND METHODS

Chemicals and reagents

TAM HCl and TDL reference standards were obtained from authenticated pharmaceutical sources. HPLC-grade methanol, water, triethylamine, and other analytical-grade reagents were used throughout the study.

Instrumentation

Chromatographic analysis was carried out by an analytical system of HPLC systems with a quaternary pump, autosampler, column oven, and ultraviolet detector. Data acquisition and processing were done using chromatography software.

AQbD framework

Analytical target profile (ATP)

The ATP was defined to achieve adequate resolution between TAM and TDL peaks, acceptable retention times, symmetrical peak shapes, and sufficient sensitivity for accurate quantification and summary results were tabulated in Table 1, and resolution responses are tabulated in Table 2.

Identification of critical quality attributes (CQAs)

Retention time, resolution, tailing factor, theoretical plates, and peak symmetry were CQAs. Normal Plot of residuals is shown in Figure 1.

Risk assessment

Potential critical method parameters (CMPs), including mobile phase composition, pH, flow rate, column

Table 1: Fit summary

Source	Sequential *P*-value	Lack of fit *P*-value	Adjusted R^2	Predicted R^2	Model Remark
Linear	<0.0001		0.9071	0.8520	
2FI	0.9786		0.8815	0.6607	
Quadra Tic	<0.0001		0.9943	0.9602	Suggested
Cubic			1.0000		Aliased

Table 2: Response 1: Resolution

Sources	Sum of square	df	Mean square	F-value	*P*-value Prob>F	Model Remark
Mean versus total	12.92	1	12.92			
Linear versus mean	1.997E-003	3	6.657E-004	5.97	0.0087	Suggested
2FI versus linear	7.333E-004	3	2.444E-004	3.41	0.0611	Suggested
Quadratic versus 2FI	1.509E-004	2	7.543E-005	1.07	0.3885	Aliased
Cubic versus quadratic	2.167E-004	3	7.222E-005	1.03	0.4527	Aliased
Residual	3.491E-004	5	6.983E-005			
Total	12.92	17	0.76			

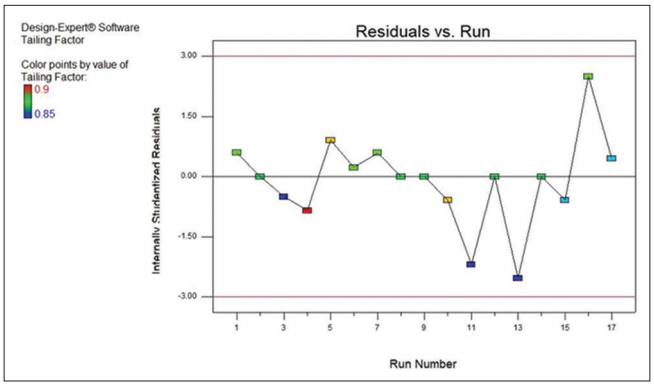

Figure 1: Normal plot of residuals for tamsulosin hydrochloride and tadalafil

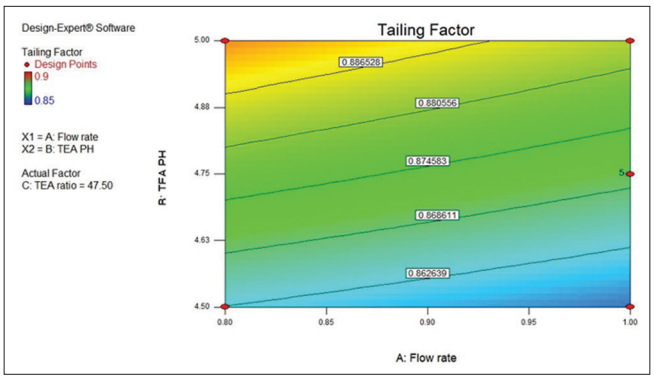

Figure 2: Predicted versus actual response for tamsulosin hydrochloride and Tadalafil

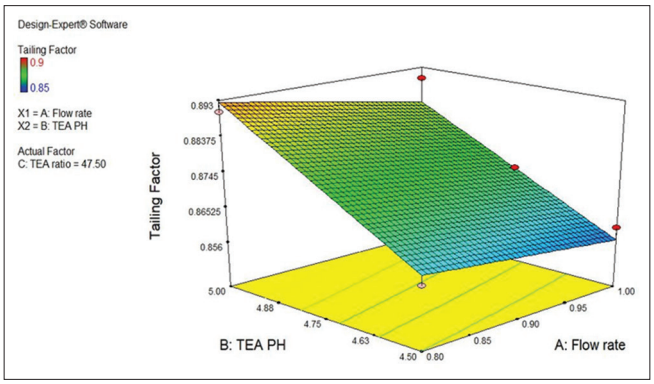

Figure 3: Resolution for tamsulosin hydrochloride and Tadalafil

temperature, and detection wavelength, were evaluated using risk assessment tools, for example, Ishikawa diagrams and failure mode and effects analysis. Responses recorded were shown in Figure 2.

Optimization of the methods and design space

Systematic experimental trials were conducted to study the effect of CMPs on CQAs. The design space was developed to guarantee the method's performance under different chromatographic conditions. Resolution for both the drugs was shown in Figure 3 and 3D Surface for both the drugs was shown in Figure 4.

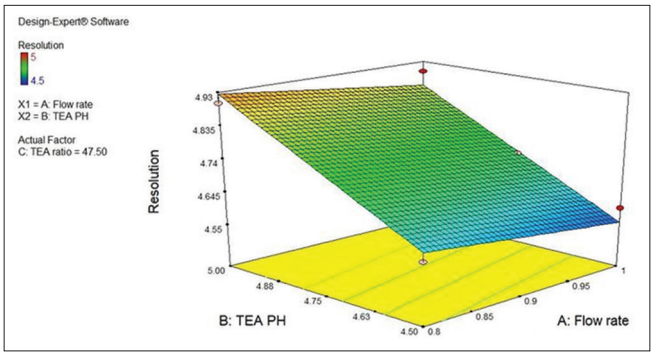

Figure 4: 3D Surface for tamsulosin hydrochloride and tadalafil

Table 3: HPLC chromatographic conditions

Parameter	Conditions
Column	C_{18} (150×4.6 mm, 3 µm)
Mobile phase	Triethylamine buffer: Methanol (45:55, v/v)
Flow rate	1.0 mL/min
Detection wavelength	243 nm
Injection volume	10 µL
Run time	10 min
Column temperature	Ambient

HPLC: High-performance liquid chromatography

Chromatographic conditions

The chromatographic analysis was performed using a RP-HPLC system equipped with a quaternary pump, autosampler, column oven, and detector, and chromatographic parameters were tabulated in Table 3.

Preparation of standard and sample solutions

Standard stock solutions of TAM and TDL were prepared separately in methanol and diluted with the mobile phase to obtain working solutions. Sample solutions were prepared from the marketed pharmaceutical formulations by the appropriate extraction and dilution procedures. Standard and sample chromatograms are shown in Figures 5 and 6.

METHOD VALIDATION (ICH Q2(R1))

System suitability

System suitability parameters were evaluated before analysis, and results are tabulated in Table 4.

Linearity

The method exhibited linearity over the concentration range of 5–30 µg/mL for TAM and 50–300 µg/mL for TDL. The correlation coefficients were >0.999 for both analytes.

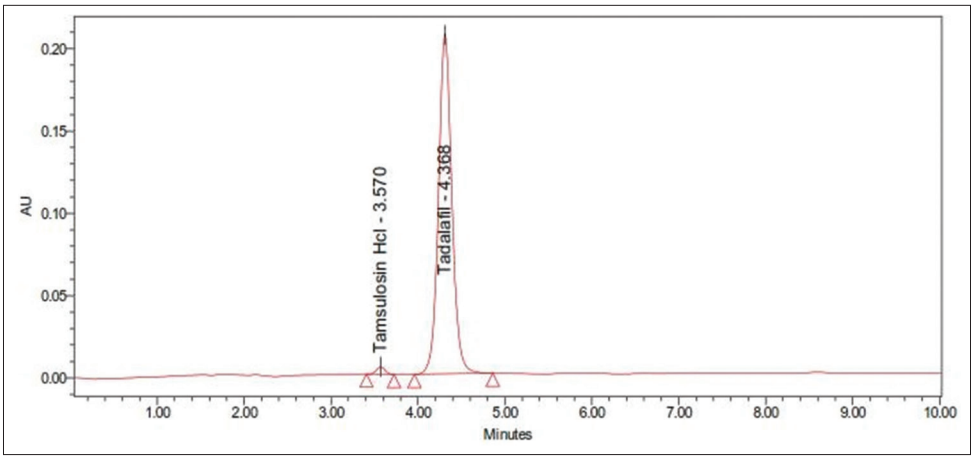

Figure 5: Chromatogram for standard

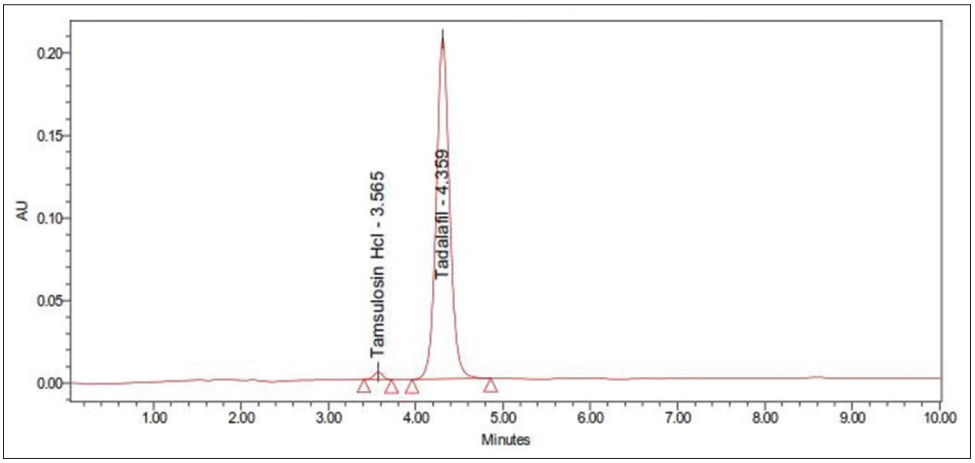

Figure 6: Chromatogram for the sample

Table 4: HPLC parameters

Parameter	Tamsulosin	Tadalafil
Retention time (min)	3.54	4.36
Tailing factor	1.03	1.07
Theoretical plates	3050	6928
Resolution	>2.0	>2.0

HPLC: High-performance liquid chromatography

Table 5: Linearity values of TAM HCl and TAD

S.No	TAM HCl		TAD	
	Concentration (µg/mL)	Area	Concentration (µg/mL)	Area
1.	1.6	3274	20	152221
2.	3.2	6748	40	336442
3.	4.8	10596	60	522884
4.	6.4	14192	80	705768
5.	8	17384	100	881536

HCl: Hydrochloride, TAM: Tamsulosin, TAD: Tadalafil

Calibration graphs plotted were shown in Figure 7, and Linearity results are tabulated in Table 5.

Precision

The precision of the method was carried out for both sample solutions as described under experimental work. Precision results are tabulated in Table 6.

Accuracy

Accuracy was assessed by recovery studies at three concentration levels (80%, 100%, and 120%), and results were tabulated in Tables 7 and 8.

Limit of detection (LOD) and limit of quantification (LOQ)

The lowest concentration of the sample was prepared with respect to the baseline noise, and measured the signal-to-noise ratio. LOD and LOQ results were tabulated in Tables 9 and 10.

Robustness

Robustness studies proved that small intentional changes in chromatographic conditions did not significantly affect

the method performance, and the method was proved to be reliable.

Stability and forced degradation studies

Forced degradation studies were conducted in acidic, alkaline, oxidation, thermal, and photolytic conditions. The developed method was able to separate the degradation product from the analyte peaks, hence its stability-indicating capability. Results of Stability studies were tabulated in Tables 11 and 12, and forced degradation results data were shown in Table 13.

Table 6: Results of precision for TAM HCl and TAD

Injection	TAM HCl area	TAD area
Injection-1	10553	529784
Injection-2	10452	512752
Injection-3	10498	522255
Injection-4	10327	522363
Injection-5	10678	512125
Injection-6	10771	522486
Average	10546.5	520294.1667
Standard deviation	159.4976489	6732.095823
%RSD	1.5	1.3

RSD: Relative standard deviation, HCl: Hydrochloride, TAM: Tamsulosin, TAD: Tadalafil

RESULTS AND DISCUSSION

The AQbD-based RP-HPLC technique allowed efficient separation of TAM and TDL with acceptable retention times and symmetrical peak shapes. The method robustness and reproducibility were assured by using the AQbD principles for the systematic optimization of chromatographic conditions.

Results from linearity studies showed that a direct proportional relationship existed between the concentration and the area of the peak over the range studied. Precision studies showed that the method is excellent repeatability with following a % relative standard deviation of <2%, which matched ICH acceptance criteria. Accuracy studies showed the method to be reliable, with recovery numbers being within acceptable limits.

The low LOD and LOQ values represented the high sensitivity of the developed method. Robustness studies were further used to validate the robustness of the method when small variations are observed in terms of

Table 7: Accuracy (recovery) data for tamsulosin HCl

% Concentration (at specification level)	Area*	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50	5279	0.8	0.79	99.27	99.05
100	10612	1.6	1.58	98.90	
150	15763	2.4	2.38	99.00	

HCl: Hydrochloride. *Area represents the mean peak area of three replicate measurements.

Table 8: Accuracy (recovery) data for tadalafil

% Concentration (at specification level)	Area*	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50	261642	10	9.86	98.58	98.85
100	527884	20	19.9	99.44	
150	784526	30	29.6	98.53	

*Area represents the mean peak area of three replicate measurements

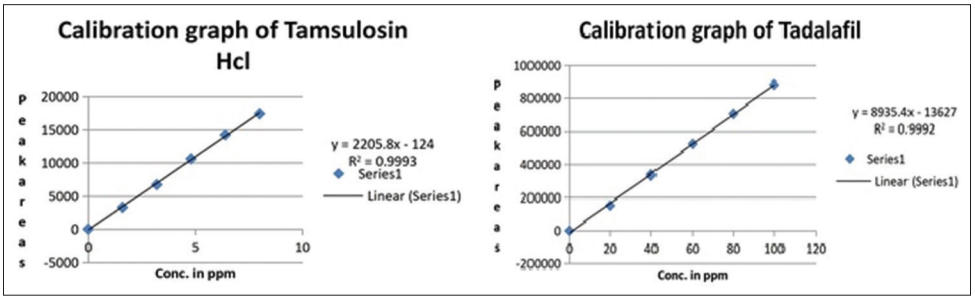

Figure 7: Calibration graphs for tamsulosin and tadalafil

Table 9: Chromatogram of TAM HCl and TAD showing LOD

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio	Conc.
TAM HCl	95	282	2.96	0.4 µg/mL
TDL	135	400	2.96	1.3 µg/mL

LOD: Limit of detection, HCl: Hydrochloride, TAM: Tamsulosin, TAD: Tadalafil, S/N ratio: Signal-to-noise ratio

Table 10: Chromatogram of TAM HCl and TAD showing LOQ

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio	Conc.
TAM Hcl	95	940	9.89	1.3 µg/mL
TDL	135	1342	9.94	4.3 µg/mL

LOQ: Limit of quantification, HCl: Hydrochloride, TAM: Tamsulosin, TAD: Tadalafil, S/N ratio: Signal-to-noise ratio

Table 11: Accelerated stability study data (40°C±2°C/75% RH±5% RH)

Time interval	Tamsulosin assay (% LC)	% Degradation	Tadalafil assay (% LC)	% Degradation	Physical appearance	Remarks
Initial (0 Month)	100.12	-	99.85	-	White capsule	Within limits
1 Month	99.48	0.64	99.02	0.83	No change	Within limits
2 Months	98.76	1.36	98.21	1.64	No change	Within limits
3 Months	97.92	2.20	97.35	2.50	Slight dullness	Within limits
6 Months	96.85	3.27	96.12	3.73	No significant change	Within limits

RH: Relative Humidity; LC: Liquid chromatography; TAM: Tamsulosin; TDL: Tadalafil.

Table 12: Long-term stability study data (25°C±2°C/60% RH±5% RH)

Time interval	Tamsulosin assay (% LC)	% Degradation	Tadalafil assay (% LC)	% Degradation	Physical appearance	Remarks
Initial	100.12	-	99.85	-	White capsule	Acceptable
3 Months	99.72	0.40	99.31	0.54	No change	Acceptable
6 Months	99.21	0.91	98.84	1.01	No change	Acceptable
9 Months	98.85	1.27	98.42	1.43	No change	Acceptable
12 Months	98.32	1.80	97.96	1.89	No change	Acceptable

RH: Relative Humidity; LC: Liquid chromatography; TAM: Tamsulosin; TDL: Tadalafil.

Table 13: Forced degradation study data

Stress condition	TAM % assay	% Degradation	TDL % assay	% Degradation	Peak purity (TAM)	Peak purity (TDL)	Remarks
Acid (0.1N hydrochloric acid, 60°C, 2 h)	93.45	6.67	90.82	9.03	Pass	Pass	Moderate degradation
Base (0.1N sodium hydroxide, 60°C, 2 h)	94.12	6.00	88.96	10.89	Pass	Pass	Significant degradation (TDL)
Oxidative (3% hydrogen peroxide, 24 h)	91.38	8.74	92.64	7.21	Pass	Pass	High degradation
Thermal (60°C, 24 h)	97.54	2.58	96.83	3.02	Pass	Pass	Mild degradation
Photolytic (ultraviolet, 24 h)	96.78	3.34	95.92	3.93	Pass	Pass	Mild degradation
Hydrolytic (water, 24 h)	98.12	2.00	97.46	2.39	Pass	Pass	Slight degradation

TAM: Tamsulosin, TDL: Tadalafil

chromatographic conditions. Forced degradation studies showed that this is a stability-indicating method, with degradation products being well-resolved from the analyte peaks.

The integration of AQbD principles enabled a holistic insight into the performance and variability of the methods, with major benefits compared to traditional methods development approaches based on trial-and-error. The established design

space required analytical consistency, meaning that the method could easily be used for routine quality control and stability studies.

CONCLUSION

A stability-indicating RP-HPLC method for the simultaneous estimation of TAM HCl and TDL was successfully developed using an AQbD approach and validated in accordance with ICH Q2(R1) guidelines. The method demonstrated excellent accuracy, precision, linearity, robustness, and sensitivity. The developed method is suitable for routine quality control and stability analysis of TAM and TDL in pharmaceutical dosage forms.

REFERENCES

1. International Conference on Harmonisation. Validation of Analytical Procedures: Text and Methodology Q2(R1). ICH Harmonised Tripartite Guideline; 2005.
2. Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs-a review. J Pharm Anal 2014;4:159-65.
3. Vogt FG, Kord AS. Development of quality-by-design analytical methods. J Pharm Sci 2011;100:797-812.
4. Snyder LR, Kirkland JJ, Dolan JW. Introduction to Modern Liquid Chromatography. 3rd ed. Hoboken, NJ: Wiley; 2010.
5. Beg S, Sharma G, Katare OP, Lohan S, Singh B. Development and validation of stability-indicating liquid chromatographic method using quality by design approach. J Chromatogr Sci 2015;53:1029-39.
6. Roopa D, Naresh.P, Gampa VK, Sravanthi B. Indicating RP-HPLC method development and validation for the simultaneous estimation of tamsulosin HCl and tadalafil in pharmaceutical dosage forms. Asian J Chem Pharm Res 2025;13:8-14.
7. Pandharinath HS, Pradeep CH, Aravinda Reddy P. RP-HPLC method development validation for the concurrent identification of Tamsulosin and Tadalafil in pharmaceutical dosage form. Int J Med Pharm Res 2025;13:136-44.
8. Bhangale C, Kalambe R, Vaditake K. Validated dissolution method for simultaneous estimation of Tamsulosin HCl and Tadalafil tablet dosage form using RP-HPLC. Bioscan 2025;20:225-33.
9. Pawar R, Naresh P, Kalva B, Kumar MR, Ravichandiran V, Ramalingam P. Analytical quality by design approach for the development of high-performance liquid chromatography method for simultaneous analysis of metformin and sitagliptin in the presence of major degradation products. J Sep Sci 2024;47:e2300605.
10. Nihal P, Mohapatra D, Manir AA, Harish V, Singh SK, Lad SU, *et al*. Reverse phase-high-performance liquid chromatography (RP-HPLC) method development and validation using analytical quality-by-design approach for determination of isoliquiritigenin in bulk and biological sample. Assay Drug Dev Technol 2024;22:409-24.
11. Devi DA, Bhavani PG. Development and validation of stability indicating UPLC method for the simultaneous estimation of triamterene and hydrochlorothiazide in combined dosage forms using quality by design approach. Future J Pharm Sci 2023;9:9.
12. Kumar A, Somasekhar V, Dhiman S, Nanjappa SH, Avlani D. An AQbD driven stability indicating HPLC method for simultaneous estimation of lamivudine, tenofovir disoproxil fumarate and efavirenz in plasma. Anal Methods 2025;17:2094-111.

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