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Implementing Analytical Quality by Design (AQbD) for the Development of a Stability-Indicating Method for Tapentadol HCl in Bulk Drug and Nasal Spray

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KEYWORDS
Tapentadol HCl, AQbD, Stability-indicating method, RP-HPLC, Nasal spray, Forced degradation, Method validation

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ABSTRACT

Purpose: To develop and validate a stability-indicating RP-HPLC method for quantifying Tapentadol HCl in bulk drug and nasal spray formulation using Analytical Quality by Design (AQbD) principles, addressing a critical gap in the literature for nasal spray formulations.

Methods: A Central Composite Design (CCD) optimized critical method parameters: mobile phase pH (4.5–5.5), flow rate (0.8–1.2 mL/min) and aqueous phase proportion (60–70%). Forced degradation studies under acid, alkaline, oxidative, thermal, and photolytic conditions assessed stability-indicating capability. Method validation followed ICH Q2(R1) guidelines.

Results: Optimized conditions—triethylammonium acetate buffer (10 mM, pH 5.0): acetonitrile (65:35, v/v) at 1.0 mL/min—achieved baseline resolution between Tapentadol and degradation products (Rs1 = 3.5, Rs2 = 2.8) with retention time of 7.738 min. Tapentadol HCl showed marked susceptibility to oxidative degradation (16.55% under 3% H_2O_2) while remaining stable under photolytic and thermal conditions. The method demonstrated excellent linearity (r^2 = 0.999 over 2–12 µg/mL), accuracy (mean recovery 99.9–100.1%), precision (%RSD < 0.5%) and sensitivity (LOD = 0.2 µg/mL, LOQ = 0.7 µg/mL). Successful application to Tapease NS Nasal Spray (label claim 225 mg/mL) yielded 99.69% recovery (%RSD = 0.38%), confirming practical utility.

Conclusion: This AQbD-driven, stability-indicating RP-HPLC method offers a robust, reliable, and regulatory-compliant approach for routine quality control and stability testing of Tapentadol HCl in both bulk drug and innovative nasal spray formulations with particular value for oxidative degradation monitoring.

INTRODUCTION

Tapentadol hydrochloride chemically (1R,2R)-3-(3-dimethylamino-1-ethyl-2-methylpropyl)-phenol hydrochloride (Fig. 1) is a synthetic opioid analgesic with a dual mechanism of action involving both µ-opioid receptor agonist and norepinephrine reuptake inhibition[1]. This unique pharmacological profile positions Tapentadol as a potent analgesic for moderate to severe pain management with a reduced incidence of gastrointestinal side effects often associated with traditional opioids[2,3]. Tapentadol HCl exhibits moderate plasma protein binding of approximately 20% and has a half-life of about 4 hours. Its oral bioavailability is in the range of 32-45%[4].

Recent advancements in drug delivery have focused on alternative routes of administration for Tapentadol HCl to overcome limitations of oral formulations such as first-pass metabolism which reduces its bioavailability to 33%[4]. Nasal spray formulations of Tapentadol HCl represent a significant innovation in this regard[2,4]. The intranasal route offers several benefits over conventional methods including rapid onset of action due to direct absorption through the highly vascularized nasal mucosa avoidance of hepatic first-pass metabolism and improved patient compliance particularly in acute pain settings or for patients experiencing dysphagia or gastrointestinal dysfunction[2]. This bypass of the gastrointestinal tract and liver can significantly enhance the bioavailability of drugs with high first-pass metabolism.

The development of robust and reliable analytical methods is paramount for quality control stability assessment and impurity profiling of pharmaceutical products especially for novel formulations like nasal sprays. Literature survey revealed various analytical techniques have been reported for the quantification of Tapentadol HCl in bulk drug, pharmaceutical formulations and biological matrices. Table no. 1.

Table 1: Summary of reported analytical methods for Tapentadol HCl

Author (Year)	Analytical Method	Purpose / Key Findings
[5]Kathirvel et al. (2013)	RP-LC (Isocratic)	Simultaneous estimation of Tapentadol and its process-related impurities in bulk and

		dosage forms; validated for stability-indicating properties.
[6]Marathe et al. (2013)	RP-HPLC	Determination of Tapentadol in bulk and tablets; observed degradation primarily under alkaline stress conditions.
[7]Lakshminarayana et al. (2015)	HPLC (Gradient mode)	Estimation of Tapentadol hydrochloride and three potential process-related impurities in bulk drug samples.
[8]Mulgund and Khutale (2021)	RP-HPLC	Rapid and sensitive quantitation of Tapentadol HCl in pharmaceutical dosage forms as per ICH guidelines.
[9]Kathirvel and Madhu Babu (2012)	HPTLC (Densitometry)	First reported high-performance thin-layer chromatographic method for Tapentadol HCl in bulk and tablet forms.
[10]Singh and Dinda (2013)	RP-HPLC	Development of a stability-indicating method for Tapentadol in tablets using a phosphate buffer and methanol mobile phase.
[11]Pandya and Joshi (2013)	Isocratic RP-HPLC	Stability-indicating assay for solid dosage forms; drug was subjected to oxidation, hydrolysis, photolysis, and thermal stress.
[12]Adluri and Kumar (2019)	LC-MS/MS	Quantification of Tapentadol in biological matrices (rabbit plasma) for bioavailability studies.
[13]Thimma Reddy et al. (2012)	RP-HPLC	Simultaneous estimation of Tapentadol and Paracetamol in combined bulk and tablet dosage forms.
[14]Sherikar et al. (2016)	LC-MS/MS	Identification and structural characterization of major oxidative degradation products.
[15]Ghemud et al. (2024)	HPTLC (Densitometry)	Developed a reproducible HPTLC method using ethyl acetate: methanol: ammonia (6:4:0.5 v/v/v). Performed forced degradation

		under six stress conditions.
[16]Gupta et al. (2019)	Dissolution & Thermal Analysis	Investigated the effect of stress on dissolution stability using both thermal and non-thermal analytical methods.
[17]Chadha et al. (2016)	RP-HPLC-UV	Isocratic stability-indicating method; performed real-time and accelerated stability testing on marketed formulations.
[18]Coulter et al. (2010)	LC-MS/MS	Developed a procedure for determining Tapentadol and its metabolite (N-desmethyl tapentadol) in urine and oral fluid.
[19]Kale and Gupta (2015)	RP-HPLC (Isocratic)	Validated a rapid method using phosphate buffer and acetonitrile (65:35); findings followed zero or first-order degradation kinetics.
[20]Jain and Basniwal (2013)	RP-HPLC-DAD	Focused on a method that avoids the use of diethylamine in the mobile phase, achieving elution at 5.34 minutes.

While these conventional analytical methods provide foundational characterization. The pharmaceutical industry increasing advocates for the implementation of the Analytical Quality by Design (AQbD) framework. AQbD is a systematic risk-based approach to analytical method development that ensures method reliability, robustness and flexibility throughout its lifecycle[21].

A stability-indicating method is critical as it accurately measures the active pharmaceutical ingredient in presence of its degradation products, impurities and excipients which is achieved by subjecting the drug to forced degradation under various stress conditions. This process intentionally generates degradation products and the method must demonstrate its ability to separate the intact drug from degradants.

Despite the established advantages of Tapentadol HCl nasal spray and the prevalence of analytical methods for Tapentadol HCl there remains a notable gap in the literature regarding the development and validation of stability-indicating analytical methods specifically for tapentadol HCl nasal spray formulations using the comprehensive AQbD framework. This gap highlights the novelty and significance of developing such a method which would ensure enhanced method reliability, lifecycle management and regulatory compliance for nasal formulation.

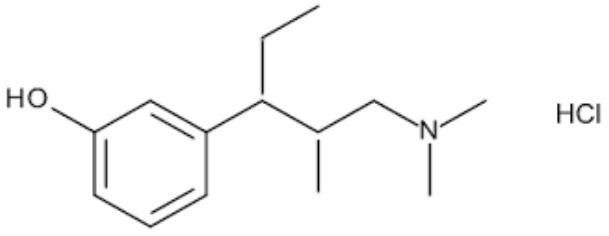


Figure 1. Chemical structure of Tapentadol hydrochloride

METHODOLOGY

This High-purity purified water was obtained using a Millipore water procured from UDCT, Aurangabad. HPLC-grade acetonitrile was sourced from Sigma Aldrich Chemicals Pvt. Ltd, Bangalore, Karnataka, India. Analytical reagent (AR) grade Triethylamine and Glacial Acetic Acid was acquired from SD Fine Chemicals Pvt. Ltd. Tapentadol Hydrochloride Nasal Spray (Tapease NS®) manufactured by Torrent Pharmaceuticals Ltd was procured from the market. While the active pharmaceutical ingredient (API) was supplied by Swapnroop Research Private Limited, Aurangabad, Maharashtra, India.

Instrumentation

Shimadzu Quaternary gradient HPLC system (P-Series) with a Shimadzu SPD-M40 PDA Detector with Kromacil C18, 4µm, (4.6

×250mm) column was used for the chromatographic separation. Lab Solution (Version DB 6.110) software was used for data acquisition and analysis. Equip-Tronics Micro Controller pH Meter Model EQ-621 was used to measure the pH of the buffer. All weighing was done on Shimadzu Model: AUW220D balance. The Stat-Ease Design Expert Version 9.0.2® software aids in the method development through QbD by designing experiments (DoE), analyzing and modelling the data and establishing a design space.

Method development

Preparation of Stock Solution

An accurately weighed 10 mg of TPD was diluted with ACN sonicated for 5 min and volume was made up to 10 mL (1000 µg/mL).

Initial Chromatographic analysis

Several mobile phase compositions were evaluated to achieve optimal separation in accordance with USP guidelines. Initial screening involved phosphate and acetate buffers at varying pH with different organic modifiers. The mobile phase consisting of triethylammonium acetate (10 mM, pH 5.0) and acetonitrile (65:35, v/v) was found to be optimal providing excellent chromatographic performance for Tapentadol. Under these conditions Tapentadol eluted with a retention time Rt of 7.738 min and exhibited a sharp, symmetrical peak (peak area: 1,114,837; tailing factor: 1.273). The method demonstrated high efficiency as indicated by a theoretical plate count of 5715. All parameters met the prescribed system suitability criteria confirming the robustness of the method for quantitative analysis (Fig. 8C).

Forced Degradation Study

Forced degradation studies were conducted on Tapentadol in accordance with ICH guidelines Q1A (R2) and Q1B to interpret its inherent stability characteristics and validate the stability-indicating power of the analytical method. The drug was subjected to a range of stress conditions including acid, base, oxidative, thermal hydrolysis and photolytic degradation with the extent of degradation controlled to approximately 5-20% to ensure the formation of relevant degradants without excessive parent drug destruction conditions detailed in Table No.1. The results indicated that Tapentadol is stable under photolytic and thermal conditions but susceptible to acid, base stress. Notably oxidative hydrolysis was identified as the most significant degradation pathway with the most substantial degradation 16.55% observed under 3% H_2O_2 for 6 hours which yielded a specific degradation products. Examination of all chromatograms confirmed that the analytical method effectively separated Tapentadol from its degradation products demonstrating its stability-indicating capability. Due to its pronounced susceptibility oxidative degradation was selected as the critical pathway for subsequent Analytical Quality by Design (AQbD) studies to ensure robust method optimization.

Table 2: Force degradation study of Tapentadol HCl

Forced degradation	Stress condition	Degradation observed
Acid hydrolysis	1N HCl at 60 °C for 12 h	02.561
	1N HCl at 70 °C for 12 h	04.520
	1N HCl at 80 °C for 12 h	06.891
Alkaline hydrolysis	1N NaOH at 60 °C for 24 h	03.124
	1N NaOH at 70 °C for 12 h	05.220
	1N NaOH at 80 °C for 6 h	07.891
Oxidative hydrolysis	1% H2O2 for 24 h	03.869
	2% H2O2 for 12 h	07.025
	3% H2O2 for 6 h	16.546
Photolytic degradation	24 h	01.391
	12 h	No degradation
Exposed to sunlight in bright sunny day	6 h	No degradation
Thermal degradation	80 °C for 12 h	02.451
	70 °C for 6 h	No degradation

Experimental Design and Optimization

The optimization phase was systematically executed using the principles of Analytical Quality by Design (AQbD) to develop a robust method capable of separating Tapentadol from its degradation products under Oxidative hydrolysis conditions.

Analytical Target Profile (ATP)

The primary goal of the method was to develop stability-indicating nature. Consequently the Analytical Target Profile (ATP) was defined to ensure the specific and reliable quantification of Tapentadol HCl in the presence of its degradation products.

Selection of Critical Method Parameters (CMPs) and Critical Quality Attributes (CQAs)

The systematic AQbD approach includes screening and optimization of CMP. It was done by the Ishikawa diagram (Fig.2) and risk assessment (Fig.3). A systematic risk assessment is performed to identify critical method parameters and critical process parameters whose variability may affect potential CQA were directly derived from the ATP. The relative risk due to each parameter was ranked as high, medium or low. High risk means those quality attributes that could have a high impact on the stability method. Low risk means those quality attributes that had a low impact on the stability method. pH of the buffer, Flow rate and Percentage of aqueous phase in the mobile phase were selected Table No3.

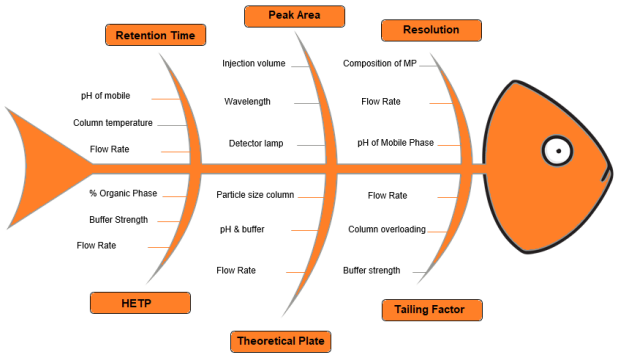


Figure 2. The Ishikawa (fishbone) diagram to identify potential variables in HPLC method development.

CQA	pH	Flow Rate	% Organic Phase	Buffer	Type of Column	Injection Volu	Temperature	Wavelength	Particle size Column
Resolution									
Tailing Factor									
Peak area									
Retention Time									
Theoretical plate									
HETP									

High Impact
Moderate Impact
Less Impact

Figure 3. Risk assessment for stability method of Tapentadol

Table 3: CMP and CQA of AQbD study

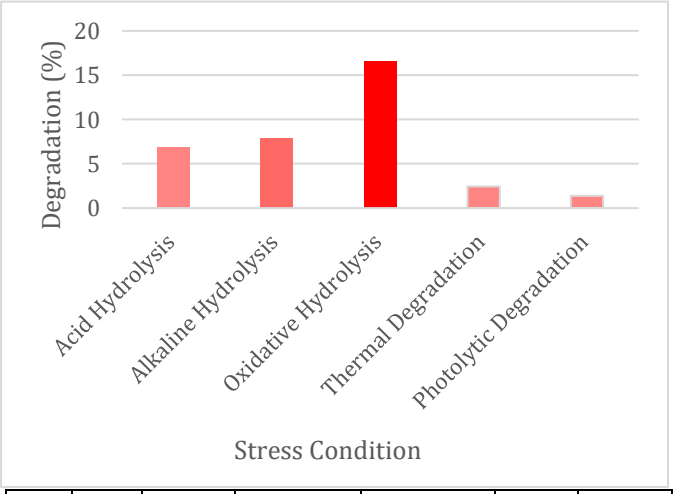
Code	Parameter	−1	0	+1
A	Buffer pH	4.5	5	5.5
B	Flow (mL/min)	0.8	1	1.2
C	% of aqueous Phase	60	65	70
Responses (CQAs) **Rs1 Resolution between Tapentadol and Degradant 1 (DP-1)** **Rs2 Resolution between Degradant 1 (DP-1) and Degradant 2 (DP-2)**				

Experimental Design and Statistical Analysis

Central Composite Design (CCD) a standard response surface methodology design was employed to systematically study the interaction and quadratic effects of the three CMPs on the two CQAs. The design consisted of total number of runs was 20 experimental runs including 8 factorial points, 6 axial points and 6 center points. The experiments were performed in a randomized order to minimize the effect of uncontrolled variables results Table No. 4.

Table 4: Design of Experiments for the optimized chromatographic parameters

Std	Run	pH of MP	Flow Rate ml/min	% Aques Phase	Rs(1-2)	Rs2(2-3)
10	1	5.84	1	65	4.2	1.8
15	2	5	1	65	3.5	2.8
1	3	4.5	0.8	60	1.8	3.5
16	4	5	1	65	3.6	2.9
7	5	4.5	1.2	70	2.1	4
18	6	5	1	65	3.5	2.8
9	7	4.16	1	65	1.5	3.8



17	8	5	1	65	3.4	2.7
5	9	4.5	0.8	70	2.9	4.8
2	10	5.5	0.8	60	3.5	1.9
20	11	5	1	65	3.5	2.8
13	12	5	1	58.3	2.8	1.2
4	13	5.5	1.2	60	2.6	1.5
19	14	5	1	65	3.6	2.9
12	15	5	1.34	65	2.5	2.5
8	16	5.5	1.2	70	3.8	2.2
14	17	5	1	71.7	4.5	3.8
6	18	5.5	0.8	70	4.8	2.7
11	19	5	0.66	65	4	3.2
3	20	4.5	1.2	60	1.2	2.8

The relationship between the factors and the responses was modeled using second-order polynomial equations. The statistical significance of the model was evaluated using Analysis of Variance (ANOVA). The quality of the fit was expressed by the coefficient of determination (R^2), adjusted R^2 and predicted R^2. The adequacy of the model was further confirmed by diagnostic plots such as residual vs. predicted plots and normal probability plots of residuals. All design generation, data analysis and optimization were performed using Design-Expert® version 12.

RESULTS AND DISCUSSION

Forced Degradation Studies:

Forced degradation studies confirmed the stability-indicating capability of the developed HPLC method for Tapentadol. The drug was most susceptible to oxidative stress showing significant degradation (16.55%) as summarized in figure 4. The method effectively separated the two oxidative degradants from the main peak leading to the selection of this pathway for subsequent robust method optimization using AQbD.

Figure 4. Degradation of Tapentadol under Various Conditions

Method Optimization using CCD:

Experimental Design and Model Fitting

The optimization of the chromatographic separation was performed using a Central Composite Design (CCD). Three critical method parameters (CMPs) were investigated: A: pH of the Mobile Phase, B: Flow Rate (mL/min) and C: Mobile Phase Ratio. The critical quality attributes (CQAs) were the resolution between the critical peak pairs namely Rs1 Resolution between Tapentadol and Degradant 1 (DP-1) and Rs2 Resolution between Degradant 1 (DP-1) and Degradant 2 (DP-2). The data was fitted to various models and ANOVA was used to select the most appropriate one for each response. For Rs1 the Quadratic model was found to be significant whereas for Rs2 the Linear model was adequate.

Interpretation:

Rs1: The model for the first resolution is highly significant ($p < 0.0004$) with an excellent R^2 of 0.94, indicating the model explains 97% of the variation in the data. The factors A (pH), B (Flow Rate) and C (% of aqueous Phase) are all highly significant. The interaction between pH and Flow Rate (AB) and the quadratic effect of Flow Rate (B^2) are also significant. The significant Lack of Fit suggests there might be some systematic variation not captured by the model but the high R^2 and significance of the model justify its use for optimization within the design space. Final Equation in Terms of Coded Factors was.

Rs1 = 3.56 + 0.8235A – 0.4245B + 0.5848C – 0.0625AB – 0.0375BC + 0.0125ABC

The perturbation and contour plot for Rs (1–2) indicates that the pH of the mobile phase (A) has the most significant influence on resolution, showing a strong positive effect as its value increases. The mobile phase ratio (C) also exhibits a positive but comparatively moderate effect on Rs (1–2). In contrast the flow rate (B) shows a negative effect where an increase in flow rate leads to a reduction in resolution. Overall the plot suggests that resolution is primarily governed by mobile phase pH followed by mobile phase composition while higher flow rates adversely affect chromatographic separation.

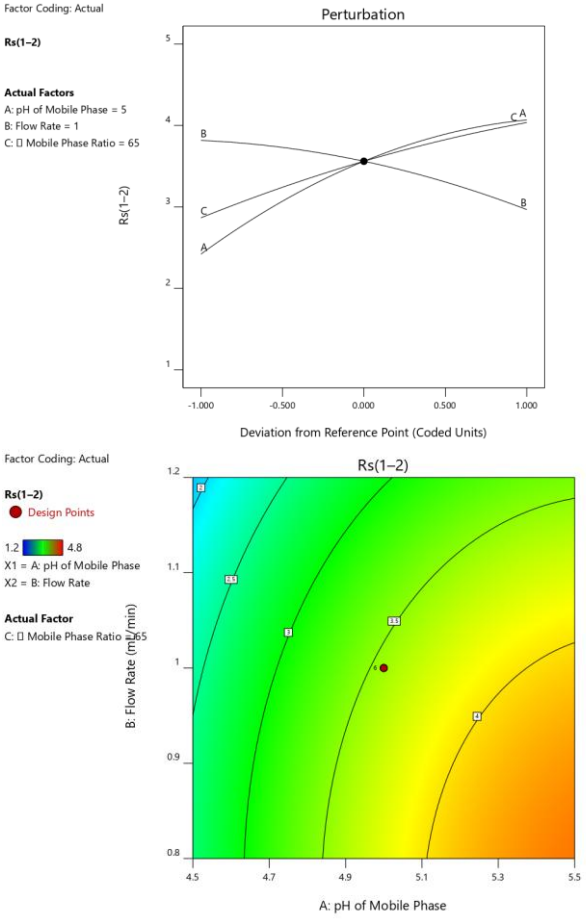


Figure 5. Design-Expert perturbation plot for SIAM of response R1

Rs2: The linear model for the second resolution is also significant ($p < 0.0001$) but with a lower R^2 of 0.80. All three linear factors are significant. The highly significant Lack of Fit indicates that a higher-order model might be more appropriate. Final Equation in Terms of Coded Factors was.

R2 = 2.79 – 0.7446A – 0.2605B + 0.6457C + 0.0750AB – 0.1250AC – 0.02250BC + 0.0158ABC

The perturbation and contour plot for Rs (2–3) demonstrates that the mobile phase ratio (C) exerts a positive influence on resolution, with an increase in C leading to improved separation between peaks 2 and 3. In contrast, the pH of the mobile phase

(A) shows a pronounced negative effect as higher pH values result in reduced resolution. The flow rate (B) also exhibits a slight negative effect on Rs (2–3). Overall the plot indicates that resolution between peaks 2 and 3 is mainly governed by mobile phase composition, while increases in pH and flow rate adversely affect chromatographic resolution.

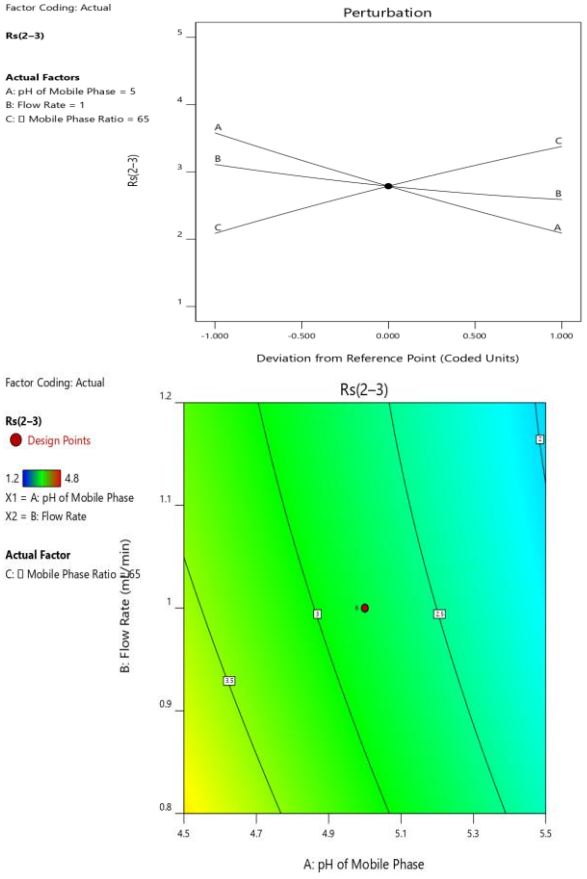


Figure 6. Design-Expert perturbation plot for SIAM of response R2

The desirability plot revealed a well-defined design space in which the pH of the mobile phase and mobile phase ratio simultaneously satisfied the resolution criteria for Rs (1–2) and Rs (2–3). Overlay contour plots demonstrated that adequate and consistent resolution was achieved within this region confirming the robustness of the method. The optimized conditions selected from the high-desirability zone ensure reliable separation and compliance with the QbD-based method development approach.

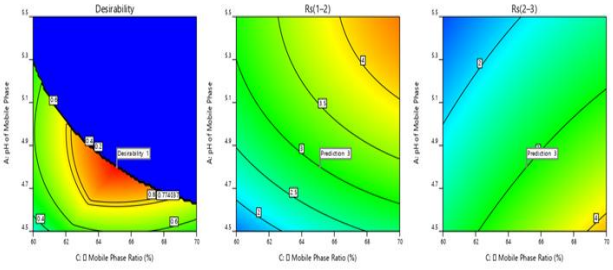


Figure 7. Overlay Contour Plot for Resolution (Rs) of Critical Peak Pairs

Method Validation:

The optimized HPLC method was validated according to ICH Q2(R1) guidelines to ensure its suitability for the intended purpose. The parameters assessed included specificity, linearity, accuracy, precision, LOD, and LOQ.

Specificity

Specificity was demonstrated by injecting a blank (the mobile phase), a placebo formulation containing all nasal spray excipients, a standard solution of Tapentadol and a stressed sample (oxidatively degraded Tapentadol). Chromatographic analysis confirmed that the blank and placebo showed no interfering peaks at the retention times of Tapentadol or its degradants (Figure 8). The standard solution showed a single sharp peak for Tapentadol while the stressed sample chromatogram clearly showed the Tapentadol peak along with well-separated two degradant peaks. These results confirm that the method is specific and stability-indicating with no interference from excipients or blank components.

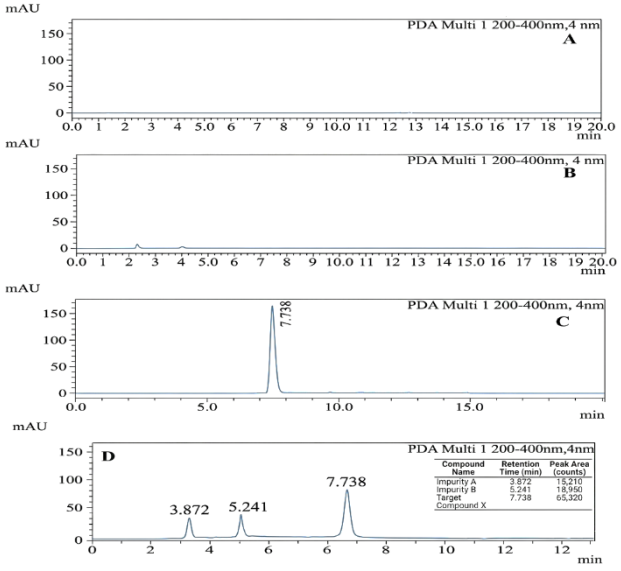


Figure 8. Chromatographic overlay demonstrating specificity: (A) blank, (B) placebo (nasal spray excipients), (C) Tapentadol standard and (D) oxidatively stressed Tapentadol sample showing separated degradant peaks.

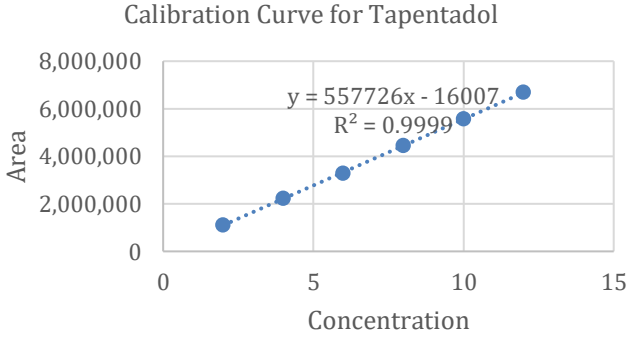


Figure 9: Linearity curve of Tapentadol HCl.

LOD and LOQ

LOD and LOQ were determined by S/N ratio that was found to be 3:1 and 10:1 respectively. The minimal amount of sample that can be detected has been identified using LOD and it was discovered

to be 0.2 µg/mL. The minimal quantity of sample that was capable of being quantified was discovered to be 0.7 µg/mL, or LOQ

Accuracy:
Accuracy was determined by injecting a concentration of 80%, 100% and 120% with each three injections. Accuracy at three different concentrations were in the limit of 97-103% so the developed method passes this parameter as shown in Table 5.

Table 5: Recovery data Tapentadol HCl

Accuracy Level	Theoretical Conc. (mg/mL)	Amount Added (mg/mL)	Amount Found (mg/mL)*	Recovery (%)	Mean Recovery (%)	% RSD
80% (n=3)	180	180.0	179.2	99.6	99.9	0.46
	180	180.0	180.5	100.3		
	180	180.0	179.8	99.9		
100% (n=3)	225	225.0	224.1	99.6	100.0	0.35
	225	225.0	225.4	100.2		
	225	225.0	224.8	99.9		
120% (n=3)	270	270.0	269.5	99.8	100.1	0.29
	270	270.0	270.8	100.3		
	270	270.0	270.1	100.0		

Precision:
Precision of the developed method was evaluated as intra-day and inter-day precision using Tapentadol standard solutions at three concentration levels (1.0, 1.5, and 2.0 µg/mL). Each level was analyzed in triplicate on the same day for intra-day precision and on three consecutive days for inter-day precision. The %RSD of peak areas was calculated and values below 2% demonstrated good repeatability and intermediate precision of the method.

Table 6: Precision Study

Concentration (µg/mL)	Peak Area 1	Peak Area 2	Peak Area 3	Mean Peak Area	% RSD
Intra-Day Precision (n = 3)					
1.0	31,250	31,420	31,180	31,283	0.39
1.5	46,890	47,050	46,730	46,890	0.34
2.0	62,430	62,610	62,290	62,443	0.26
Inter-Day Precision (n = 3)					
1.0	31,283	31,560	31,410	31,418	0.44
1.5	46,890	47,210	46,950	47,017	0.35
2.0	62,443	62,780	62,590	62,604	0.27

Analysis of marketed formulation:
The developed and validated AQbD-based stability-indicating method was applied to determine the Tapentadol HCl content in the marketed nasal spray formulation (label claim: 225 mg/mL). For sample preparation, an accurately measured volume (1.0 mL) of Tapease NS Nasal Spray was transferred into a 100 mL volumetric flask and diluted to volume with mobile phase to obtain a stock solution of 2250 µg/mL; the 100% test solution (225 µg/mL) was used for assay determination. The assay results showed that the amount of Tapentadol HCl found in the marketed formulation was 224.3 ± 0.42 mg/mL, corresponding to 99.69% of the label claim with a % RSD of 0.38% (n = 3)

Table 7: Assay Results of Tapease NS Nasal Spray (225 mg/mL)

Parameter	Observation
Label claim	225 mg/mL
Amount found (mg/mL)	224.3 ± 0.42
Assay (% of label claim)	99.69%
Standard deviation (SD)	0.42
% RSD (n = 3)	0.38%

RESULTS AND DISCUSSION

An Analytical Quality by Design (AQbD) approach was applied to optimize chromatographic conditions for separation of Tapentadol HCl from its oxidative degradation products. A three-factor three-level Central Composite Design (CCD) was employed with pH of mobile phase (A), flow rate (B mL/min) and percentage of aqueous phase (C) as independent variables while resolution between Tapentadol and degradant 1 (Rs1) and resolution between degradant 1 and degradant 2 (Rs2) were selected as critical quality attributes (CQAs). Resolution was strongly affected by mobile phase pH and percentage of aqueous phase; higher pH (5.5) combined with increased aqueous phase content (70%) produced maximum resolution (Rs1 = 4.8, Rs2 = 4.8), whereas lower pH (4.5) with reduced aqueous phase (60%) resulted in decreased resolution (Rs1 as low as 1.2, Rs2 as low as 1.5) due to poor separation and peak overlapping. Flow rate showed a negative effect on both Rs1

and Rs2 with higher flow rates (1.2 mL/min) leading to reduced resolution due to insufficient analyte-column interaction. Retention and separation efficiency were predominantly governed by mobile phase composition and pH; increasing the aqueous phase proportion from 60% to 70% significantly improved resolution between critical peak pairs while higher flow rates reduced retention times but compromised resolution. Response surface and contour plot analyses revealed that optimal separation with baseline resolution for both critical peak pairs was achieved at moderate pH (4.8–5.2), controlled flow rate (0.9–1.1 mL/min), and aqueous phase content between 63–67%. Based on predefined acceptance criteria (Rs1 > 2.0, Rs2 > 2.0), the Method Operable Design Region (MODR) was established; overlay contour plots identified a robust operating space at mobile phase pH 4.8–5.2, flow rate 0.9–1.1 mL/min, and aqueous phase proportion 63–67%, within which consistent resolution and acceptable separation were obtained. The optimized chromatographic conditions were selected as Triethylammonium acetate buffer (10 mM, pH 5.0): acetonitrile (65:35, v/v) at a flow rate of 1.0 mL/min. Under these conditions the experimentally achieved resolution Rs1 was 3.5 and Rs2 was 2.8 with a retention time of 7.738 min for Tapentadol, closely matching predicted values (Rs1 = 3.56, Rs2 = 2.79) and confirming the validity of the AQbD approach. Forced degradation studies revealed that Tapentadol HCl was stable under photolytic and thermal conditions but susceptible to acidic, alkaline, and particularly oxidative hydrolysis, with 16.55% degradation observed under 3% H_2O_2 for 6 hours, and the method effectively separated all degradants from the parent peak, confirming its stability-indicating capability. Method validation according to ICH Q2(R1) guidelines demonstrated specificity (no interference from blank or placebo, peak purity > 0.9999), linearity (r^2 = 0.999 over 2–12 µg/mL), accuracy (mean recovery 99.9–100.1% at 80%, 100%, and 120% levels with %RSD 0.29–0.46%), precision (intra-day %RSD 0.26–0.39%, inter-day %RSD 0.27–0.44%), and sensitivity (LOD = 0.2 µg/mL, LOQ = 0.7 µg/mL). The developed method was successfully applied to the marketed formulation Tapease NS Nasal Spray (label claim 225 mg/mL) yielding an assay value of 224.3 ± 0.42 mg/mL (99.69% of label claim, %RSD = 0.38%, n = 3) confirming its suitability for routine quality control and stability studies of Tapentadol HCl in bulk drug and nasal spray formulations.

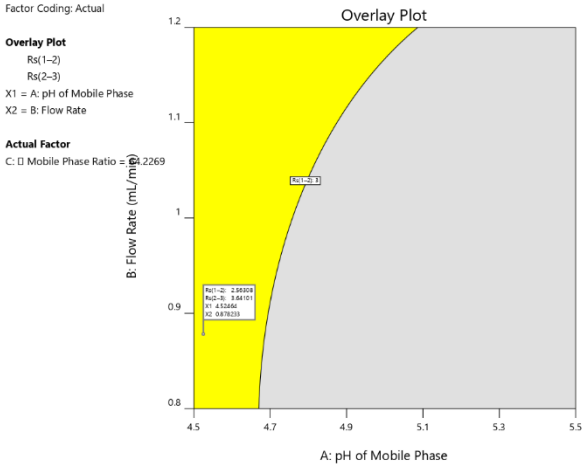


Figure 10. Overlay plot depicting MODR for Tapentadol HCl

CONCLUSION

An AQbD-driven RP-HPLC method was successfully developed and validated for stability-indicating quantification of Tapentadol HCl in bulk drug and nasal spray formulation. Central Composite Design optimized critical parameters (pH 5.0, flow 1.0 mL/min, 65% aqueous phase), achieving excellent resolution (Rs1 = 3.5, Rs2 = 2.8). Forced degradation confirmed susceptibility to oxidative stress with all degradants well separated. Validation per ICH Q2(R1) demonstrated specificity, linearity (r^2 = 0.999), accuracy (99.9–100.1%), precision (%RSD < 0.5%), and sensitivity (LOD 0.2 µg/mL). The method was successfully applied to Tapease NS Nasal Spray (99.69% label claim), confirming its reliability for routine quality control and stability studies.

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