

A QbD based RP-HPLC Method Development and Validation for Quantification of Pregabalin Capsules

ABM Mahfuz ul Alam^{1,2*}, Nilufar Nahar¹, M Iqbal Rouf Mamun¹, Mohammad Shoeb¹

¹Department of Chemistry, University of Dhaka, Dhaka, Bangladesh

²Quality Operations Department, ACI Health Care Limited, Treepordi, Sonargaon, Narayanganj, Bangladesh,
Email: mahfuz301175@gmail.com

Abstract: This study emphasizes the pivotal role of Quality by Design (QbD) in the development of pharmaceutical methods, with a particular focus on risk assessment to ensure consistent quality. The research showcases the creation of a precise and practical HPLC method for Pregabalin Capsules, developed using QbD principles. This optimized method, designed through a systematic Design of Experiment approach, provides a robust and cost-effective solution for pharmaceutical analysis, promoting the consistent quality required within predefined specifications. The method employs a Neucleosil C-18 column (150 mm x 4.6 mm, 5 μ M) and employs isocratic elution with a mobile phase composed of Acetonitrile and Phosphate Buffer (pH 6.8) in a 20: 80 v/v ratio. Key method parameters include a flow rate of 0.9 mL/min, a column oven temperature set at 40°C, an injection volume of 20 μ L, and UV detection at 210 nm. Rigorous validation following ICH Q 2 (RI) and USP <1225> guidelines ensures the method's reliability, with assessments of parameters such as limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, and robustness. The method's exceptional sensitivity, selectivity, efficiency, precision, accuracy, and cost-effectiveness make it an optimal choice for pharmaceutical analysis of Pregabalin Capsules.

Keywords: HPLC, Quality by Design (QbD), Pregabalin, Quantification, Lyrica, Chromatography, Pharmaceutical Formulations, In-vitro Analysis, Method Development, Generic Drug, Method Validation

1. Introduction

Traditional method development can be time-consuming and inefficient, leading to challenges in risk management for analytical methods. In response, the pharmaceutical industry is exploring innovative approaches, such as Analytical Quality by Design (AQbD).

Quality by Design (QbD) is a methodical approach that places a strong emphasis on comprehending both product and process, while integrating scientific principles and risk management to improve product quality and regulatory adaptability. In the jurisdiction of pharmaceutical development, QbD extends its reach to encompass analytical methods, advocating for the creation of resilient and flexible methods throughout their lifecycle. The principles of Analytical QbD align closely with those of Process QbD, highlighting the importance of early assessment of method robustness and ruggedness to avoid the need for substantial redevelopment efforts [1-5]. This research employs QbD to optimize an HPLC method for the analysis of Pregabalin in pharmaceuticals.

Pregabalin, with its chemical name (3S)-3-(aminomethyl)-5-methylhexanoic acid, is a derivative of gamma-aminobutyric acid (GABA). This compound functions as a calcium channel blocker and serves the dual role of an anticonvulsant and an anti-anxiety agent. Pregabalin, similar to gabapentin, exerts its therapeutic effects by binding to the $\alpha 2\delta$ subunit of voltage-gated calcium channels [6]. This mechanism of action is key to its efficacy in managing seizures and anxiety-related conditions. Moreover, Pregabalin exhibits analgesic properties, making it valuable in the treatment of neuropathic pain and fibromyalgia. The chemical structure of Pregabalin is illustrated in Figure 1.

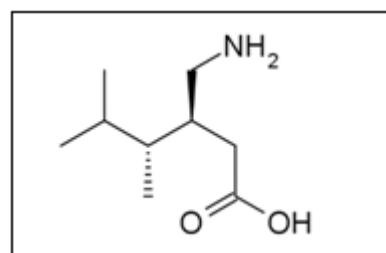


Figure 1: Chemical Structure of Pregabalin [(S)-3 (aminomethyl)-5-methylhexanoic acid]

Based on the literature review, it is evident that numerous analytical methods using HPLC and spectroscopy techniques exist [7-10], but there are no established methods for analyzing formulated dosage forms in any pharmacopoeia. This absence of methods in pharmacopoeias leaves products not included in these references without appropriate means to assess their quality. Furthermore, the previously published Pregabalin methods are notably complex. Notably, a significant number of manufacturers have already introduced Pregabalin Capsules in Bangladesh, as the country is exempt from patent laws under the World Trade Organization (WTO) agreement, valid until 2033 [11]. Consequently, there is a pressing need to develop a stability-indicating assay method with shorter analysis times, achievable through the Quality by Design (QbD) approach.

To address this need, a chromatographic separation method for Pregabalin was developed using the principles of Analytical Quality by Design (AQbD). The method was optimized through a Design of Experiment (DOE) approach, considering four critical factors: buffer pH, acetonitrile concentration, flow rate, and oven temperature, while evaluating three essential responses: retention time, asymmetry, and theoretical plate. This optimization process

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was statistically analyzed using tools such as counter plots, Pareto charts, and interaction plots, leading to the development of an optimized method.

The validated method adhered to the requirements outlined in ICH Q2 (R1) [12] and USP <1225> [13], encompassing assessments of parameters like limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, and robustness. The validated method exhibited exceptional sensitivity, selectivity, speed, precision, accuracy, and cost-effectiveness. This makes it a highly suitable choice for pharmaceutical industries to analyze Pregabalin Capsules.

Furthermore, the validated method was utilized to quantify the active content in locally produced major drugs in various dosage forms and in innovator's drugs. It was also employed to conduct in-vitro dissolution comparisons between local non-pharmacopeial drugs and innovator's drugs, using the *f1* and *f2* metrics to assess dissimilarity and similarity [14]. The results demonstrated in-vitro dissolution similarity across all examined brands, indicating comparable potency to the innovator's drugs. With only one exception, all the drugs manufactured in Bangladesh met international standards, ensuring their effectiveness for treating various health conditions, both locally and in overseas markets.

2. Experimental

Chemicals, Reagents, and Samples

High-performance liquid chromatography (HPLC) Grade Acetonitrile, anhydrous sodium dihydrogen orthophosphate,

Phosphoric Acid, Sodium Hydroxide, Hydrogen Peroxide, Sodium Metabisulfite, Sodium Acetate Trihydrate and Glacial Acetic Acid, were sourced from the local distributor of Scharlau, Spain. Hydrochloric Acid and Disodium Hydrogen Orthophosphate Dihydrate were obtained from Active Fine Chemicals in Bangladesh. Phosphoric Acid and Acetonitrile were procured from Merck, Germany. The innovator drug LYRICA® by Pfizer was acquired through Pharmaceutical Buyers Inc, USA, while various local brands of Pregabalin Capsules were obtained from reputable pharmacies in Bangladesh.

Equipment

The experimental apparatus included a Gradient HPLC system manufactured by Dionex in the USA and equipment from Waters Corporation, which featured both UV Detector and PDA detectors. Additional equipment comprised a Sartorius Weighing Balance, a Labconco Glassware washing machine, a Hanson Vision G2 Elite 8 Dissolution Tester, a pH meter from Mettler in Switzerland, a Thermal oven, and an Ultrasonic Water bath produced by Memmert in Germany. Laboratory-grade water was generated using the Labconco water purification system. These tools and equipment were employed in method development, validation, and the analysis of market samples.

HPLC Method Development by AQbD Approach:

To develop an HPLC method using the Analytical Quality by Design (AQbD) approach, the following eight sequential steps (Table 1) were followed.

Table 1: Steps to develop analytical method as per QbD approach

Step	Activities	Data to Generate / Evaluate
Step 1:	Selection of Analytical Method Target Profile	Determine Analytical Method Target Profile Qualitatively by Using Fish Bone Diagram - Method Parameters/Factors/Variables (e.g. Mobile Phase, Column, pH of Buffer, Column Temperature, Injection volume, Particle Size of HPLC Column, Analyte type, Diluent type, Detector etc.) - Method Performances/Attributes /Responses (e.g. Accuracy, Precision, Robustness, Retention time (RT), Theoretical plate (N), etc.)
Step 2:	Literature Search	Data were collected for the intended analyte through literature survey: - Molecular structure and molecular weight - pKa - to understand whether the molecule is acidic, basic or neutral - Type of functional group - Presence of Chromophore - Log P (partition coefficient) - Solubility - Available Method
Step 3:	Identify method parameters by method scouting	Define method parameters based on: - Analyte physiochemical Properties - Physiochemical Properties of Mobile Phase - Stationary phase compatibility with Analyte and Mobile Phase
Step 4:	Identify predictive critical method parameters by Qualitatively/ Quantitatively	- Qualitative Method: o Discard predictive values like column length, Injection volume o Include unpredictable items like pH of buffer, mobile phase composition, Flow Rate - Quantitative Method o Risk Assessment for high risk items which may have high impact on response e.g. pH of buffer, mobile phase composition, Column oven temperature, Flow Rate etc.
Step 5:	DoE for Multivariate Interaction Study	- Set up Critical Method parameters to the responses over a range
Step 6:	Screening and Optimization	- Scientific understanding of relation between quantities of input variables (CMP) and output response which will show considerable effect on method

Step	Activities	Data to Generate / Evaluate
		performance (Interaction plot, surface plot, Contour plot etc.) - Define method operable design space
Step 7:	Define Final operable Method and Execute Method Validation	- Stability indicating-study specificity (force degradation) - Accuracy, Precision, Robustness, Solution Stability etc.
Step 8:	Monitoring and Lifecycle Management	- Evaluate Market products and RLD - Evaluate Change requirements for improvements

Assessment of Experimental Outcomes and Determining Optimal Method Conditions

The primary objective was to investigate the impact and interactions of four distinct variables: the pH of the buffer solution, flow rate, percentage of acetonitrile, and column

temperature on various responses, specifically, retention time, peak asymmetry, and theoretical plate. To achieve this, an experimental design was created to span the range of each variable, as detailed in Table 2.

Table 2: Factors and Their Levels Selected for DoE Studies

Molecules Factors	pH of Buffer		% Acetonitrile		Oven Temperature OC		Flow Rate (mL/min)		Response Retention Time, Asymmetry, Theoretical Plate
	Min	Max	Min	Max	Min	Max	Min	Max	
Pregabalin	6.0	7.0	10	50	25	45	0.8	1.5	

Assessing the true effects of all four variables on the three target responses would typically require a substantial number of studies. To streamline this process and expedite the discovery of interaction patterns in a scientifically rigorous manner, a design of experiments (DoE) was formulated, utilizing Microsoft Minitab Software 16.1.1. The DoE was based on a comprehensive full factorial

experimental design, with each block containing two (02) center points.

This Design of Experiment (DoE) resulted in the identification of 18 distinct experimental combinations, as outlined in Table 3. These combinations were subsequently used to formulate various mobile phases by employing different combinations of buffer and organic solvent.

Table 3: Experimental Design for Preliminary Assessment of Pregabalin in Method Development

S. N.	Factors				Response		
	Buffer pH	% Acetonitrile	Flow Rate	Oven Temp	RT	Asymmetry	Plate Count
1	7	10	1.5	25	2.04	1.08	2804
2	6.5	30	1.15	35	1.66	1.14	660
3	6	50	1.5	25	0.97	1.51	484
4	6	10	0.8	45	3.79	1.11	6609
5	6	50	1.5	45	1.02	1.16	338
6	6	10	1.5	25	2.07	1.10	2819
7	6	10	0.8	25	3.78	1.15	5667
8	6	50	0.8	45	1.31	1.01	980
9	6.5	30	1.15	35	1.65	1.22	726
10	7	10	0.8	45	3.76	1.07	6889
11	7	50	1.5	45	0.72	1.19	224
12	6	50	0.8	25	1.74	1.85	839
13	7	50	0.8	25	1.35	1.11	880
14	7	10	1.5	45	2.05	1.04	3202
15	7	50	1.5	25	0.97	1.57	442
16	7	10	0.8	25	3.72	1.15	5536
17	6	10	1.5	45	2.08	1.04	3301
18	7	50	0.8	45	1.23	1.44	331

The goal was to optimize the HPLC method for Pregabalin analysis by comprehensively studying these parameters using C18, 150 mm x 4.6 mm, 5 µM HPLC column with 10 µL injection volume using HPLC equipped with PDA detector, column oven and auto injector.

Evaluation of experimental results and selection of final method conditions

The responses to variations in individual factors were scrutinized through various statistical models, including Interaction plots, pareto charts, and contour plots (Figure 1a-h). These analyses were employed to gain a comprehensive understanding of the genuine impact of the four variables on

responses. Subsequently, an optimized chromatographic method was chosen for validation as follows:

HPLC Column: C18, 150 mm x 4.6 mm, 5 µM
Mobile Phase: Acetonitrile: Phosphate Buffer pH 6.8 (20: 80)

Flow Rate: 0.9 mL/min

Detection: UV detector was set at 210 nm

Injection Volume : 20 µL

Column Oven Temperature: 40°C

Retention time: 3.4 minutes

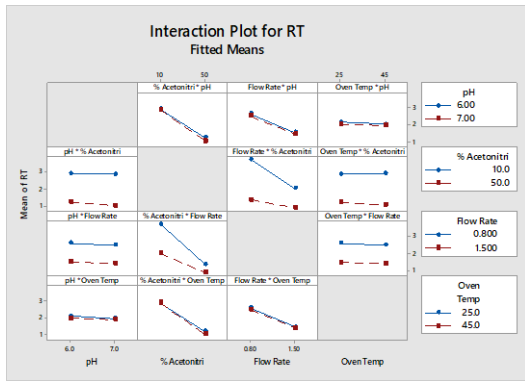


Figure 1 (a): Interactions Retention Time vs Variables

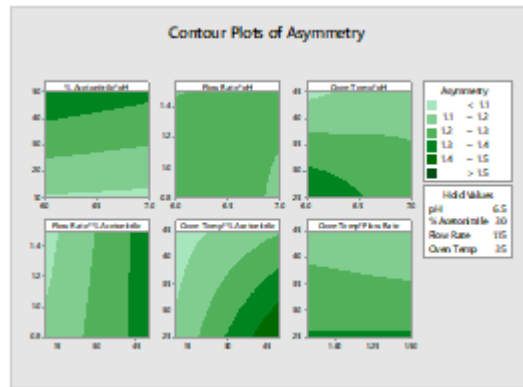


Figure 1 (e): Contour Plots of Asymmetry Over Variables

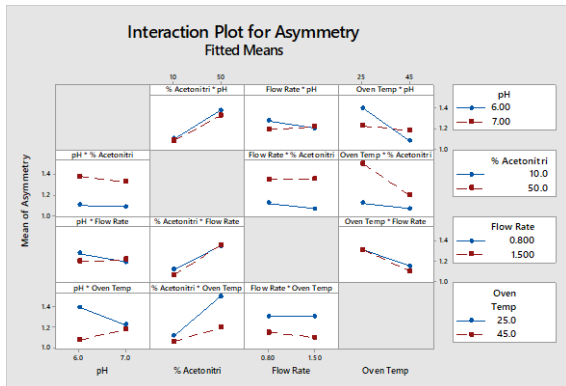


Figure 1 (b): Interactions Asymmetry vs Variables

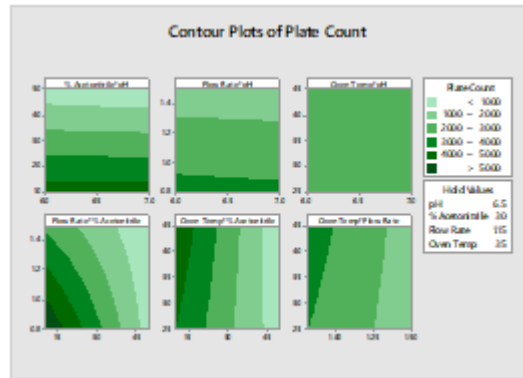


Figure 1 (f): Contour Plots of Plate Count Over Variables

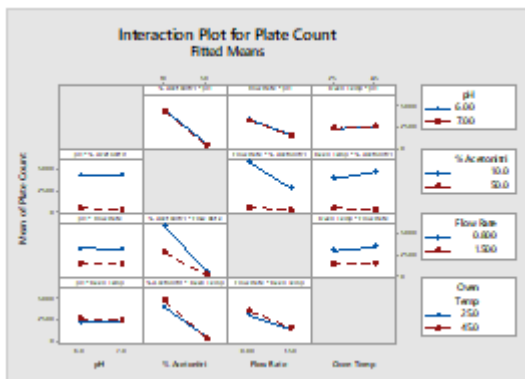


Figure 1 (c): Interactions Plate Count vs Variables

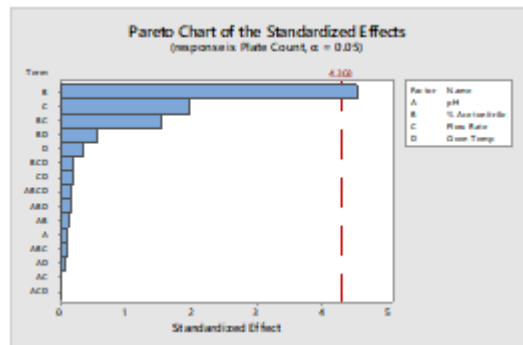


Figure 1 (g): Standardized Effects against response Plate Count

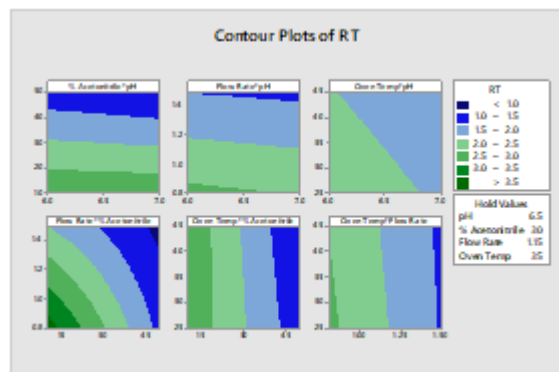


Figure 1 (d) Contour Plots of Retention Time Over Variables

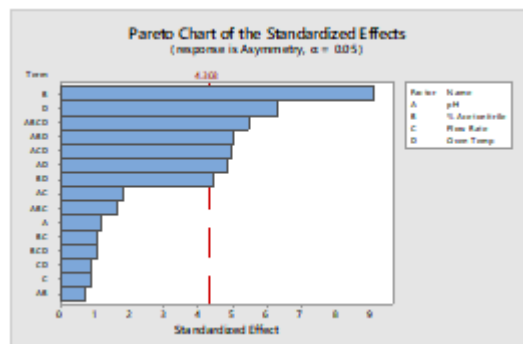


Figure 1 (h): Standardized Effects against response Asymmetry

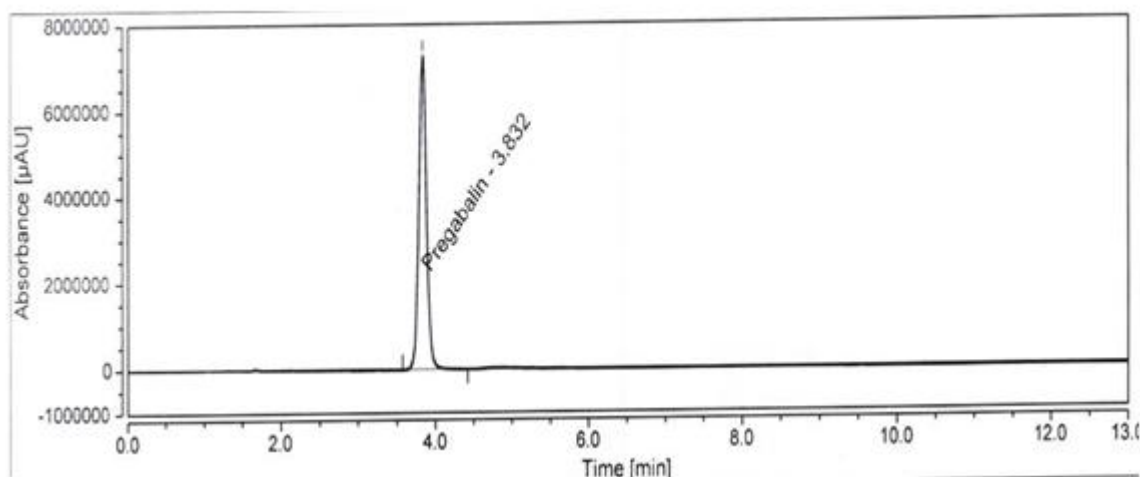


Figure 2: Reference Standard Chromatogram of Pregabalin

Method Validation

System Suitability:

The system suitability test was implemented on a representative chromatogram (Figure 2) to verify several parameters. These included theoretical plates, which were 7295, peak asymmetry, measured as 1.08, and the % RSD (Relative Standard Deviation) for six replicate injections for both area and retention time, which were 0.34% and 0.02% respectively.

Specificity:

Specificity was confirmed by analyzing placebo and formulated tablet under unstressed and stressed conditions to ensure no interference from expected components. Placebo showed no interference with the analyte peak. Stressed samples were injected into the HPLC system with a Photodiode Array Detector. Sample is mostly stable except with peroxide (Table 4). Peak purity was 997, indicating no interference from degradants, establishing the method as stability indicating.

Table 4: Summary of Specificity

Test Condition	Placebo Interface at Main Peak	Peak Purity by PDA	Control Sample (%)	Water Hydrolysis (40°C/2 Hours) (%)	Thermal Stress (80°C/48 Hours) (%)	Acid Stress (1.0 N HCl/40°C/2 Hours) (%)	Base Stress (1.0 N NaOH/40°C/2 Hours) (%)	Peroxide Stress (3% H2O2/40°C/2 Hours) (%)
Pregabalin	No	997	99.9	99	100	99	95	32

Linearity:

The calibration curve generated for Pregabalin exhibited linearity across the concentration range of 0.8 to 1.2mg/mL, as depicted in Fig. 2 and Table 5. The correlation coefficient, typically determined from the plot of peak area against concentration (Figure-3), was found to be 0.9997.

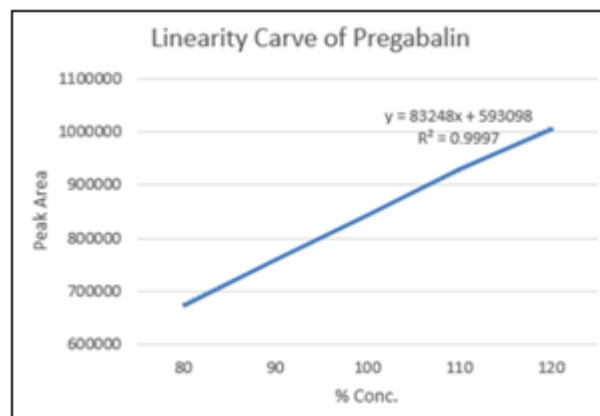


Figure 3: Linearity Curve of Pregabalin

Level	Peak Area
80%	674706
90%	759898
100%	844331
110%	928764
120%	1006515
Correlation Coefficient	0.9997

Precision:

The % RSD for repeatability in the measurement of pregabalin at the 80%-120% concentration (0.8 to 1.2mg/mL) over six replicates was determined to be less than 0.13%, Mean recovery 99.3% for interday, intraday precision was 0.72% with mean recovery 99.4%. This affirms the precision of the developed method.

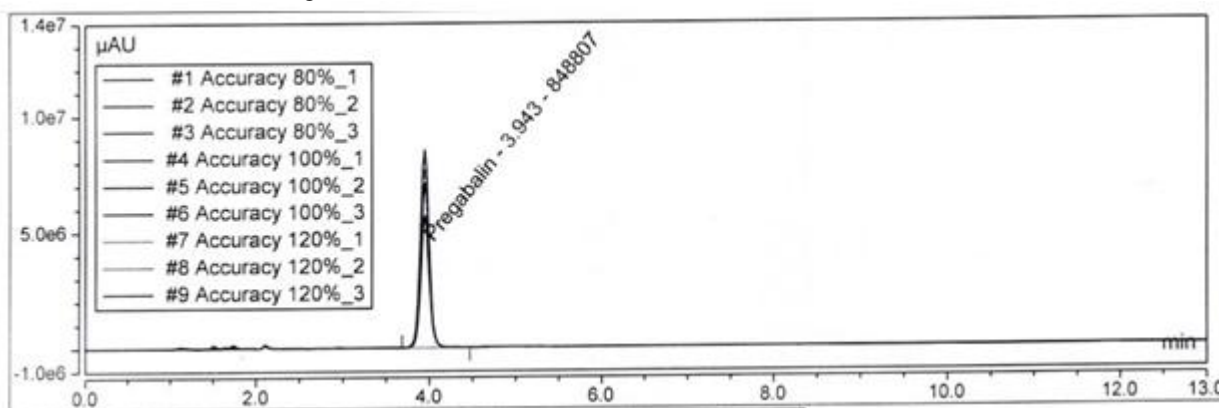
Accuracy:

Accuracy assessment was conducted through a recovery study. Sample solutions were created by spiking at three

levels: 80%, 100%, and 120%. The % recovery data, as obtained through the proposed HPLC method, is presented in Table 6. The % recovery falling within the range of 98-102% validates the accuracy of the developed method in accordance with the ICH Q2 (R1) guidelines.

Table 6: Recovery Study

Level	80%	100%	120%	Mean	% RSD
Pregabalin	100	100	99	99.7	0.57

**Figure 4:** An example Chromatogram of Accuracy of Pregabalin

Robustness and Ruggedness Studies:

The impact of altering the mobile phase ratio (Buffer: Organic Phase), detector wavelength, pH of the buffer/aqueous phase, mobile phase flow rate, and column temperature on the retention time, peak area, and asymmetry of the primary analyte was investigated using sample 1 mg/mL concentrations. No significant alterations in responses were observed with variations in these method factors, confirming the robustness of the methods (RSD 0.13%).

Table 7: Outcome of Robustness

Parameter	Variations	Pregabalin	
		RT	Peak Area
Flow Rate	+2.00%	3.2	849384
	-2.00%	3.9	842764
Elution Solvent ratios	+2%	3.7	848807
	-2.00%	4	847983
Detector Wavelength	+3nm	3.8	847653
	-3nm	3.5	848649
Temperature	+2.0°C	3.4	849739
	-2.0°C	3.4	849498
pH	+ 0.2	3.4	848569
	-0.2	3.4	847859

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The Limit of Detection (LOD) and Limit of Quantification (LOQ) for Pregabalin, calculated using the Dionex HPLC Chromeleon Software, were established as 3.5 ppm and 8.7 ppm, respectively.

Solution and Mobile Phase Stability:

Both test and standard solutions were left on the benchtop at room temperature for 2 days. Upon analysis against a fresh standard solution after 24 hours, the result was 0.6%, and after 48 hours, it was 0.82%. These results indicate that there were no significant changes in the stability of the sample solution for up to 48 hours.

Filter Study:

Samples and standard solutions were prepared and filtered through various filters, including PTFE 0.45 micron, 0.22 micron, and Nylon 0.45 micron filters. The filtered solutions were then analyzed using the established chromatographic methods. Results were assessed for differences in area obtained with different filters. The % RSD of the difference in area was found to be 0.27%, indicating no significant variation in results. The method is deemed suitable with all these filters for both qualitative and quantitative analyses.

Table 8: Filter Study

Type of Filter	PTFE 0.45	PTFE 0.22	Nylon 0.45	% RSD
Pregabalin	839872	844331	841098	0.27

Assay Content of Marketed Products:

The established methods were effectively utilized to assess the identification and assay values of various prominent brands available in the Bangladesh market. The chromatographic separations were notably clear, ensuring no interference from the excipients. The products from all manufacturers tested fell within the target acceptance range, as detailed in Table 9.

Table 9: Assay of Marketed Product

Name of Molecule	% Assay Value						Remarks
	Innovator	Mfg1	Mfg2	Mfg3	Mfg4	Mfg5	
Pregabalin	98.8	99.2	100.2	100.4	99.3	98.6	All results are good

Dissolution and In Vitro Dissolution Comparison

The dissolution assessment for five major brands of Pregabalin Capsules available in Bangladesh was conducted using USP Apparatus II (Paddle) at 75 rotations per minute with 0.06 N HCl at 37°C. The analysis, employing the

developed method, indicated favorable dissolution results for most brands. In vitro dissolution tests were performed in comparison with the Reference Listed Drug (RLD) at time points 10 mins, 20 mins, 30 mins, and 45 mins. The results, evaluated in terms of f_1 (1.2-11.7) and f_2 (48.6-65.3) values,

revealed a 60% similarity, signifying that the major tested products. brands can be considered as bioequivalent to the branded

Table 10: Dissolution Test of Marketed Generics & Innovator

Sample	RLD	Mfg 1	Mfg-2	Mfg-3	Mfg-4	Mfg-5	Remarks
Pregabalin 150 mg Capsules	92 (90-95)	103 (98-106)	103 (101-106)	100 (95-104)	99 (98-101)	96 (90-103)	All brands have good and comparable results with RLD

Table 11: In-vitro Comparison of Marketed Generics against Innovator (Lyrica)

Name of Product	f1/f2	Mfg-1	Mfg-2	Mfg-3	Mfg-4	Mfg-5
Pregabalin 150 mg Capsules	f1	9.09	11.73	11.14	6.74	1.17
	f2	53.91	48.58	49.84	60.02	65.27

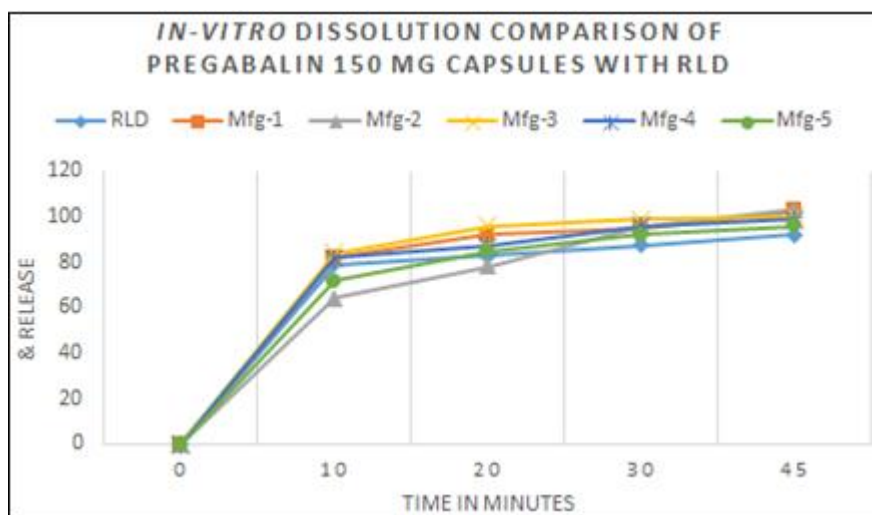


Figure 5: In-vitro dissolution comparison of different local brands of Pregabalin 150 mg Capsules with RLD

3. Discussion

A quality-by-design HPLC method has been developed for the estimation of Pregabalin in pharmaceutical formulations. Through risk assessment, the analytical target product profile identified key parameters as retention time, theoretical plates, and peak asymmetry for Pregabalin analysis by HPLC. Critical quality attributes affecting the target product profile were determined as mobile phase composition, pH of the buffer solution, flow rate, and oven temperature.

A full factorial design with Minitab Software 16.1.1, involving four factors and three responses with two center points per block, resulted in 18 independent runs. Variability in column selection, instrument configuration, and injection volume was carefully controlled. The quality-by-design approach successfully developed the HPLC method for Pregabalin was with HPLC Column: C18, 150 mm x 4.6 mm, 5 μ M, Mobile Phase: Acetonitrile: Phosphate Buffer pH 6.8 (20: 80), Flow Rate: 0.9 mL/min, Detection: UV detector set at 210 nm, Injection Volume: 20 μ L, Column Oven Temperature: 40°C. This method resulted Pregabalin peak at a retention time: 3.4 minutes

Validation of the method demonstrated satisfactory results for system suitability, accuracy, precision, robustness, linearity, sample stability, filter effect, LOD, and LOQ. The method was successfully applied to evaluate major generic brands of Pregabalin Capsules in the Bangladesh market and found comparable to the innovator product.

4. Conclusion

Analytical Quality by Design (QbD) principles were applied in the development of an HPLC method for Pregabalin. To identify the optimal system and establish the final design space, a multivariate study involving crucial process parameters—specifically, the combination of mobile phase composition, buffer pH, flow rate, and oven temperature at different levels—was conducted. Interrelationships were explored and optimized using Design of Experiment Software in Minitab 16.1.1. This approach deepened the understanding of factors influencing chromatographic separation, ensuring methods meet their intended purposes and facilitating the development of chromatographic optimization for future use.

All validated parameters were found to be within acceptable criteria. The validated method for determining Pregabalin was established as linear, precise, accurate, specific, robust, and rugged. The QbD methodology provided a deeper understanding of method variables, reducing the likelihood of failure during method validation and transfer. The automated QbD method development approach, utilizing Minitab software, yielded a more robust method in less time compared to manual development. Statistical analysis indicates the method's reproducibility, selectivity, accuracy, and robustness. This method is intended for further use in routine analysis for quality control in the pharmaceutical industry and has demonstrated the ability to distinguish marketed products, including comparability with the innovator product.

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