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Development and Validation of a Reliable HPLC Method for Chlorpromazine Analysis

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Abstract- ***The present study aimed to develop and validate a simple, accurate, precise, robust, and cost-effective reverse-phase high-performance liquid chromatography (RP-HPLC) method for the quantitative estimation of chlorpromazine in bulk drug and tablet dosage forms in accordance with ICH guidelines. Chromatographic separation was achieved using a C18 column (250 × 4.6 mm, 5 µm) with an optimized mobile phase consisting of acetonitrile and water (40:60, v/v) adjusted to pH 3.5, at a flow rate of 1.0 mL/min and UV detection at 260 nm. The developed method exhibited excellent linearity over the concentration range of 10–60 µg/mL with a correlation coefficient (R^2) of 0.9899. Validation studies demonstrated satisfactory accuracy (98.2–102.68% recovery), precision (%RSD < 2%), specificity, robustness, and system suitability. The method showed high sensitivity with low limits of detection (LOD) and quantification (LOQ), making it suitable for routine pharmaceutical quality control. Additionally, a Quality by Design (QbD)-based robustness assessment employing Central Composite Design (CCD) was performed to evaluate the influence of critical method parameters, including pH, mobile phase composition, and flow rate, on critical quality attributes. Statistical analysis using ANOVA confirmed the significance and reliability of the optimized chromatographic conditions. The developed RP-HPLC method is simple, reproducible, environmentally conscious due to reduced solvent consumption, and well suited for routine estimation of chlorpromazine in pharmaceutical formulations.***

Keywords: Chlorpromazine, RP-HPLC, Method Development, Method Validation, Quality by Design (QbD), Central Composite Design (CCD), ICH Guidelines, Pharmaceutical Analysis, Robustness, Quality Control, Reverse Phase High-Performance Liquid Chromatography, Analytical Method Validation

I. INTRODUCTION

Chlorpromazine is a first-generation antipsychotic medication widely used in the treatment of psychiatric disorders such as schizophrenia, bipolar disorder, and severe behavioral problems. It works by blocking dopamine receptors in the brain, thereby reducing symptoms like hallucinations, delusions, and agitation. Chlorpromazine is also used for nausea, vomiting, and chronic hiccups in certain cases. It is available in various formulations, including tablets, injections, and syrup.

Despite its therapeutic efficacy, chlorpromazine has certain limitations, such as variable pharmacokinetics, side effects, and stability concerns. These factors highlight the need for robust and precise analytical methods to ensure its quality and safety in pharmaceutical formulations.

A systematic approach to pharmaceutical development emphasizes understanding and controlling the processes involved in the manufacture and testing of drugs. Applying these principles to HPLC (High-Performance Liquid Chromatography) method development for chlorpromazine offers several benefits:

- Robustness and Reliability:

Traditional HPLC methods may lack robustness, leading to variability in results. A systematic approach ensures the development of a method that performs consistently under a wide range of conditions.

- Regulatory Compliance:

Regulatory agencies like the FDA encourage approaches that enhance the reproducibility and

reliability of analytical methods. This can simplify regulatory approvals and audits.

- Enhanced Method Understanding:

This approach emphasizes identifying key attributes and parameters that affect method performance. This leads to a deeper understanding of the method and its optimization.

- Cost-Effectiveness:

By preemptively identifying and mitigating risks, this approach reduces the likelihood of method failure, minimizing the need for rework and associated costs.

- Improved Stability and Sensitivity:

Chlorpromazine is prone to degradation under certain conditions. An effectively developed HPLC method can ensure high sensitivity and specificity for detecting impurities and degradation products.

- Adaptability:

Pharmaceutical formulations may vary due to changes in excipients or manufacturing processes. A method developed with thorough understanding is more adaptable to such variations without requiring complete redevelopment.

- Optimization of Chromatographic Conditions:

Through systematic experimentation (e.g., Design of Experiments - DoE), the optimal combination of factors such as mobile phase composition, flow rate, column temperature, and pH can be identified for achieving the best separation and peak resolution.

Considerations for HPLC Method Development:

- Challenges with Solvents:

Traditional HPLC methods use large volumes of organic solvents, which are often toxic, volatile, and non-biodegradable. These solvents contribute to various operational difficulties and disposal challenges.

- Regulatory Requirements:

Regulations mandate minimizing waste and adopting more effective practices in laboratories and industries.

- Health and Safety:

Exposure to certain solvents poses health risks to laboratory personnel. Methods that utilize safer alternatives can reduce such risks.

- Cost Savings:

Reducing solvent consumption and implementing solvent recycling practices can lower operational costs over time.

- Resource Management:

The global supply of certain solvents like acetonitrile is limited, and exploring and adopting different alternatives can help manage dependence on finite resources.

- Company Objectives:

Many pharmaceutical and chemical companies aim to achieve various operational targets, and implementing updated HPLC methods aligns with these goals.

- Improved Method Efficiency:

Approaches that lead to faster methods with better performance and reduced sample preparation can save time and resources.

II. RATIONALE/HYPOTHESIS

Rationale/Hypothesis:

The study is motivated by the need to develop a robust, accurate, and efficient analytical method for the estimation of chlorpromazine in bulk and tablet formulations. Conventional High-Performance Liquid Chromatography (HPLC) methods often face limitations such as the use of hazardous solvents, lengthy analysis times, and variable performance, leading to inefficiencies and potential regulatory challenges.

By focusing on method optimization, this study aims to overcome these drawbacks by improving sensitivity, specificity, and reproducibility while ensuring practicality for routine pharmaceutical analysis. The proposed method seeks to enhance analytical performance, reduce operational costs, and streamline the quantification process for

chlorpromazine, ensuring reliable results in both bulk and tablet formulations.

Key Hypotheses:

- Improved Method Robustness:

Systematic optimization of chromatographic conditions (e.g., mobile phase composition, flow rate, column selection) will result in a more reliable and reproducible HPLC method for chlorpromazine estimation.

- Enhanced Analytical Performance:

Refinement of detection parameters (e.g., wavelength, injection volume) will improve sensitivity and specificity, enabling precise quantification of chlorpromazine in pharmaceutical samples.

- Cost and Time Efficiency:

Optimization of the method will reduce solvent consumption, shorten analysis time, and lower operational costs without compromising accuracy.

- Regulatory Compliance and Practical Applicability:

The developed method will meet pharmacopeial standards and be easily adaptable for routine quality control in pharmaceutical laboratories.

By addressing these aspects, the study aims to establish a high-performing, cost-effective, and user-friendly HPLC method for chlorpromazine analysis.

III. AIM & OBJECTIVES

Aim:

To develop and validate a precise, accurate, sensitive, and cost-effective HPLC method for the quantification of chlorpromazine in pharmaceutical formulations in accordance with ICH guidelines.

Objectives:

1. Development of the Analytical Method:
   - To design an HPLC method that provides high sensitivity, accuracy, and precision for the quantification of chlorpromazine in bulk and tablet formulations.
   - To ensure the method effectively separates chlorpromazine from its impurities and degradation products.

2. Validation of the Method:
   - To validate the method as per ICH (International Council for Harmonization) guidelines, focusing on parameters such as linearity, specificity, accuracy, precision, robustness, and system suitability.

3. Sustainability Assessment:
   - To evaluate the environmental impact of the developed method using green metrics such as solvent usage, waste generation, and energy consumption.

4. Regulatory and Practical Compliance:
   - To ensure the developed method meets regulatory requirements for pharmaceutical quality control.
   - To provide a cost-effective and scalable solution for routine quality testing in industrial settings.

5. Applicability to Real Samples:
   - To apply the developed method to analyze chlorpromazine content in commercial tablet formulations and validate its practicality for routine pharmaceutical analysis.

IV. LITERATURE REVIEW

Author	Journal Name	Year	Points
Fadhel SR et, al.	J Biochem Tech	2018	The mobile phase consists of water and acetonitrile. The pH of the mobile phase is adjusted to optimize separation. pH adjustment can improve peak shape and resolution. A buffer is often added to maintain a stable pH. The mobile phase composition and pH are critical for effective HPLC analysis.

P.Shetti et, al.	E-journal of Chemistry	2010	Column: C-18 (250x4.6 mm) - Mobile phase: Water:Acetonitrile - Elution mode: Isocratic - Flow rate: 1.0 mL/min - Detection: UV at 254nm
Vunnava Prathyush et,al.	International Journal of Research Publication and Reviews	2023	QbD is applied to drug development through a systematic approach. It involves designing and developing formulations and manufacturing processes. The method used includes risk assessment, design of experiments (DoE), and control strategy development. This approach ensures quality is built into the product, reducing variability and defects. QbD facilitates regulatory flexibility and continuous improvement
Jessy Shaji et,al.	International Journal of Pharmaceutical Sciences Review and Research	2012	From this article, we learn about the key validation parameters such as linearity, accuracy, precision, specificity, robustness, LOD, LOQ, and range. Each parameter has defined acceptance limits based on regulatory guidelines. For example, accuracy should be within 98–102%, precision often requires %RSD less than 2%, and linearity should have a correlation coefficient (r^2) above 0.999. These parameters ensure the method is reliable, reproducible, and suitable for its intended purpose.

V. EXPERIMENTAL

MATERIALS AND REAGENT

Chlorpromazine (CPZ) (having a purity level of 99.98.%) of pharmaceutical grade was kindly supplied as gift samples by Ajanta Pharma, (Mumbai, India). High-performance liquid chromatography (HPLC) grade methanol, acetonitrile and water were obtained from Merck Specialties Pvt. Ltd. And Research lab Pvt. Ltd., based in India. All of the solvents used in the study were purchased from Research lab Pvt. Ltd., all of which are of analytical grade.

INSTRUMENTATION

HPLC (Thermo–Ultimate 3000 manufactured by Shimadzu LC-20 HPLC system with UV

INDIA equipped with the Lab Solution software having HPLC column C18 (250 x 4.6mm, and particle size of 5 µm) was used for analysis purposes, wavelength of 260 nm used. The HPLC system consisted of the following components: an isocratic or quaternary solvent delivery module, a gradient solvent delivery module, an online degasser, a column thermostat, an auto sampler, and a UV detector. Data analysis and interpretation were performed using the Lab Solution software. The separation was achieved using gradient elution. In addition, an Analytical Balance (WENSAR Analytical Balance), a pH meter (LABMAN instruments (LMPH10)), were used in this study.

1) UV Analysis of Drug.

Preparation of Solution

The CPZ (100 mg) was accurately and precisely weighed using an analytical balance showing a capacity of (0.1 mg to 200 g), and transferred to a 100 mL volumetric flask, volumes were made up to the mark with methanol to obtain a solution containing 1000 µg/ml. Methanol was selected due to the highest solubility achieved during saturation solubility studies. To achieve concentrations ranging from 2 to 10 µg/mL, the operational solutions were mixed with methanol and diluted

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2) Assay

- Preparation of standard stock solutions, and working standard solution

The CPZ (100 mg) was accurately and precisely weighed using an analytical balance showing a capacity of (0.1 mg to 200 g), and transferred to a 100 ml volumetric flask, volumes were made up to the mark with methanol to obtain a solution containing 1000 µg/ml. Methanol was selected due to the highest solubility achieved during saturation solubility studies. The solution was then passed through a 0.45 µm membrane filter paper, which was later utilized for analysis.

- Preparation of Mobile Phase

The optimized mobile phase comprised acetonitrile and water mixture in a ratio of 40:60 v/v. As the pka of CPZ is 9.3 it is a weak base, hence it shows good solubility and stability at pH 3.5 (basic substances stable in an acidic environment). As we are using a green chemistry approach, fewer organic solvents were used and adjusted with a glacial acetic acid. This mobile phase provided good resolution and perfect peak. The mobile phase was sonicated for 10 minutes, followed by passing through a 0.45 µm membrane filter. Table 1 shows the Selection of optimized flow rate, mobile phase, and Ph

Table 1. Selection of optimized flow rate, mobile phase, and pH

Parameter	Method condition	CPZ (Retention time)	%RSD (Peak area)	%RSD (Retention time)
Flow rate(ml/min)	0.9	3.9	0.94	1.50
	1.0	3.6	0.64	1.08
	1.1	4.1	1.11	1.04
Mobile phase (v/v) ACN-Water	30:70	3.9	0.94	1.50
	40:60	3.6	0.64	1.08
	50:50	4.1	1.11	1.04
pH	3.0	3.9	0.94	1.50
	3.5	3.6	0.64	1.08
	4.0	4.1	1.11	1.04

- Preparation of sample solution

The 20 tablets crushed, and equivalent to 100 mg, 200 mg weighed(100 mg tablet contains 50 mg CPZ) dissolved in a 100 ml volumetric flask using methanol, further by pipetting 1mL and diluting to 10 mL to prepare a concentration of 100 µg/ml followed by filtration using a 0.45µm nylon syringe filter before injecting to the instrument.

(If a 100 mg chlorpromazine tablet is supposed to contain 100 mg of chlorpromazine hydrochloride, but analysis shows it contains 50 mg, the actual drug content is only 50% of the labelled claim. To calculate how much of this tablet would be required to provide the equivalent of 100 mg of chlorpromazine, you can use the formula:

Required Amount=Desired Amount/Actual Drug Content Fraction

Where:

- Desired Amount = 100 mg
- Actual Drug Content Fraction = 50/100=0.5

Required Amount=100/0.5=200 mg

% Assay = Area of Sample/ Area of Standard) x Weight of standard/dilution x further dilution x dilution / weight of sample x further dilution x potency (1)

$$= \frac{161360}{162008} \times \frac{100}{1} \times \frac{1}{100} \times 100$$

$$= 0.9960 \times 100 \times 1$$

$$= 99.60\ \%$$

3)Validation of the developed method

The method was validated for linearity, accuracy, precision, system suitability, robustness, the limit of detection (LOD), and the limit of quantification (LOQ) as per the ICH guideline [7].

• Linearity and Range

The serial dilutions of CPZ ranging from 10 to 60 µg/ml were made, and linearity was determined [8]. The graph was plotted by taking the area under the curve on the y-axis and concentration on the x-axis. Meanwhile, the coefficient of correlation (R2) also concluded [9–11].

• System suitability test
A total number of 6 replicas of 20 µg/ml were injected. Factors considered for assessment include the tailing factor, theoretical plate, and %RSD determined [11].

• Intra-day and inter-day precision
The three different concentrations of CPZ (10, 30, 60 µg/ml) were selected to calculate the precision of inter-day. Similarly, the precision of intra-day was evaluated by assessing the same solutions in a triplicate manner, beginning from the start (morning), middle (afternoon), and end (evening), and percentage RSD was calculated. The results for Inter-day and Intraday variation studies are shown in (Table 4) [12-14].

%RSD = (Standard Deviation/Mean) × 100
Requirement: <2

• Accuracy (Recovery)
The accuracy of the method was performed by recovery studies. To determine the accuracy of the method, CPZ was added at concentrations of 25%, 50%, and 75% of the target concentration of 50 µg/ml.(Table 5) The resulting peak area was compared with the CPZ standard solution (12.5, 25, 37.5 µg/ml) peak area and the % recovery of the total drug was calculated using a formula. An acceptance criterion for recovery was set between 98% to 102%. The formula for calculating percent recovery is (Found concentration / Total concentration) x 100 [15].

ACCURACY (%Recovery) = (Measured Conc./Theoretical Conc.) × 100
Range: 98-102%

• Robustness
The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and indicates its reliability during normal usage. The small variations were done concerning flow rate (± 0.1), pH (± 0.5), and % mobile phase composition (± 2%) (Table 6) [16]. The impact of these adjustments on parameters like retention time and peak areas was assessed in terms of % R.S.D. values. In contrast, the A QbD approach employed triplicate, utilizing 6 linear points, 6 middle points, and 8 factorial points, with 20 batches as provided by Design Expert software version 13. Factors such as pH, % organic content, and flow rate were considered, and for target responses retention time (R.T), peak area (P.A), theoretical plate (T.P), and tailing factor (T.F) were considered. The Design Expert software demonstrated the % contribution of every factor followed by Analysis of Variance (ANOVA). By this statistical method, it was easy to calculate significant differences between the means of three or more groups. Additionally, perturbation plots, 3D response surface plots, and contour plots, overlay plots were generated for graphical representation, and optimization of the method 13,19.

• Specificity
Specificity was evaluated to determine any influence of solvent, and excipients used in the formulation on the method's efficacy. A total number of six injections each from standard drug and placebo was used and the resultant chromatograms were matched to determine the specificity of the method [11].

• LOD and Limit of quantitation LOQ
The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were determined by using the formulae as LOD = 3.3 (SD)/S and LOQ = 10 (SD)/S, where S represents of slopes of the calibration curve and SD is calculated using values of y-intercepts of regression equations [15].

VI. RESULT AND DISCUSSIONS

1)UV Analysis of Drug

Concentration (µg/ml)	2	4	6	8	10
Absorbance	0.201	0.370	0.562	0.760	0.938

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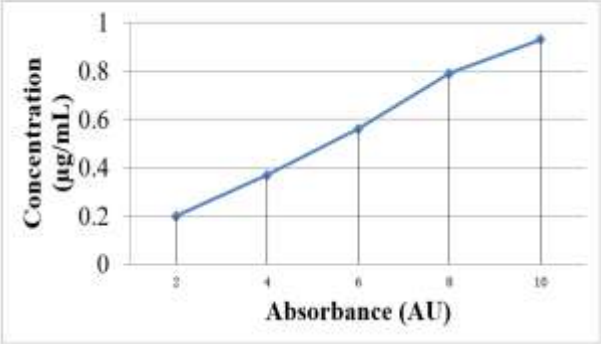
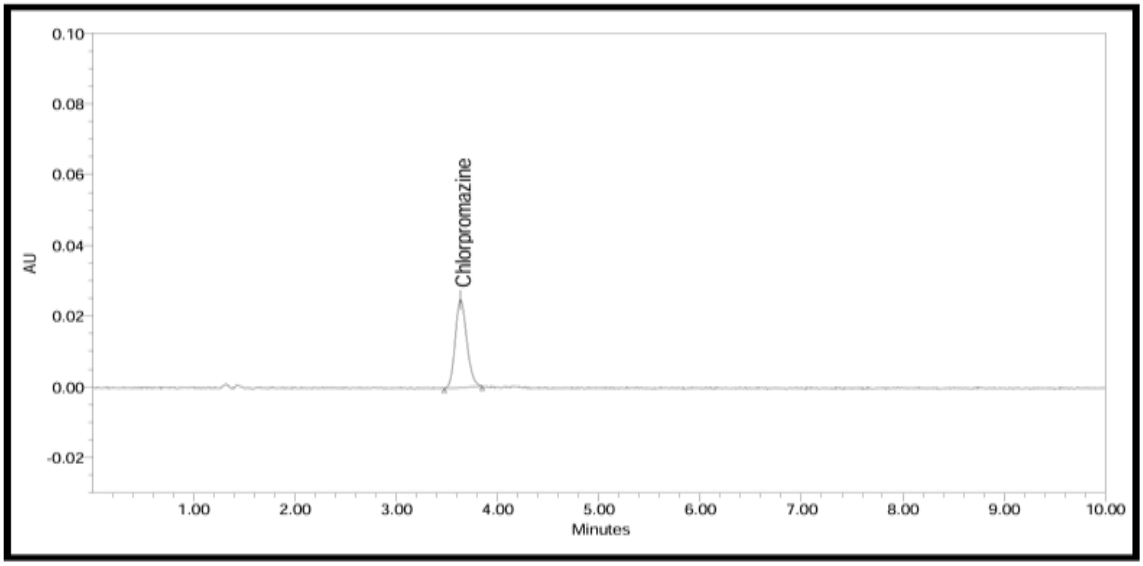


2) Assay

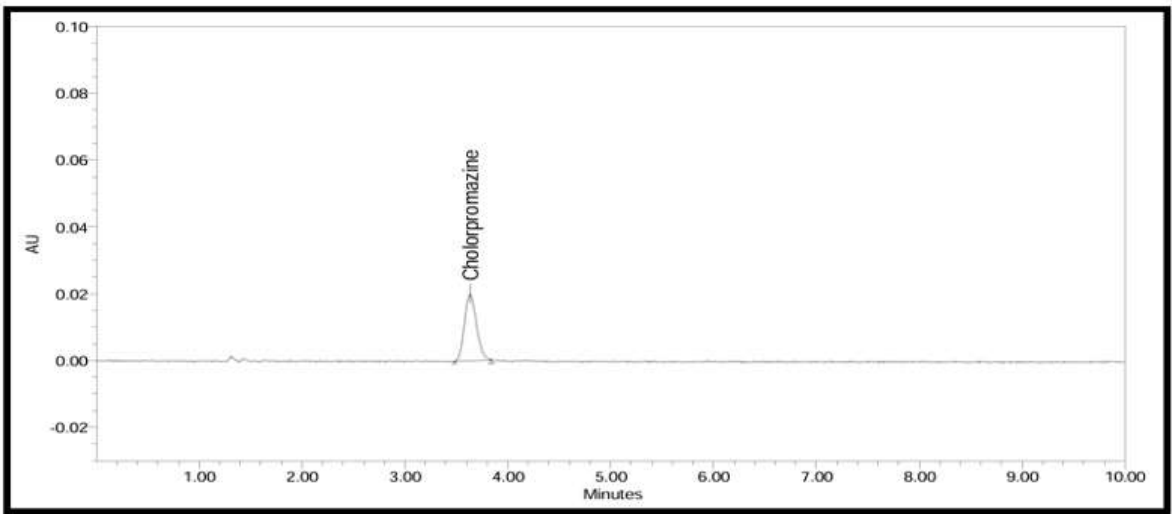
- Standard solution:

1000 □g/mL solution injected in triplicate at R.T 3.637, found area 162008 and a theoretical plate of 4654.76 with the tailing factor of 1.05 and the other two areas found were 162006, and 162010, Thus 162008 ± 2 is the mean with standard deviation.



- Sample solution:

A Chlorpromazine tablet equivalent to 100 mg was diluted with methanol (1000 □g/mL solution) injected in triplicate at R.T 3.632, and the found area was 161359,161361,161360. Thus 161360 ± 1 mean with standard deviation.



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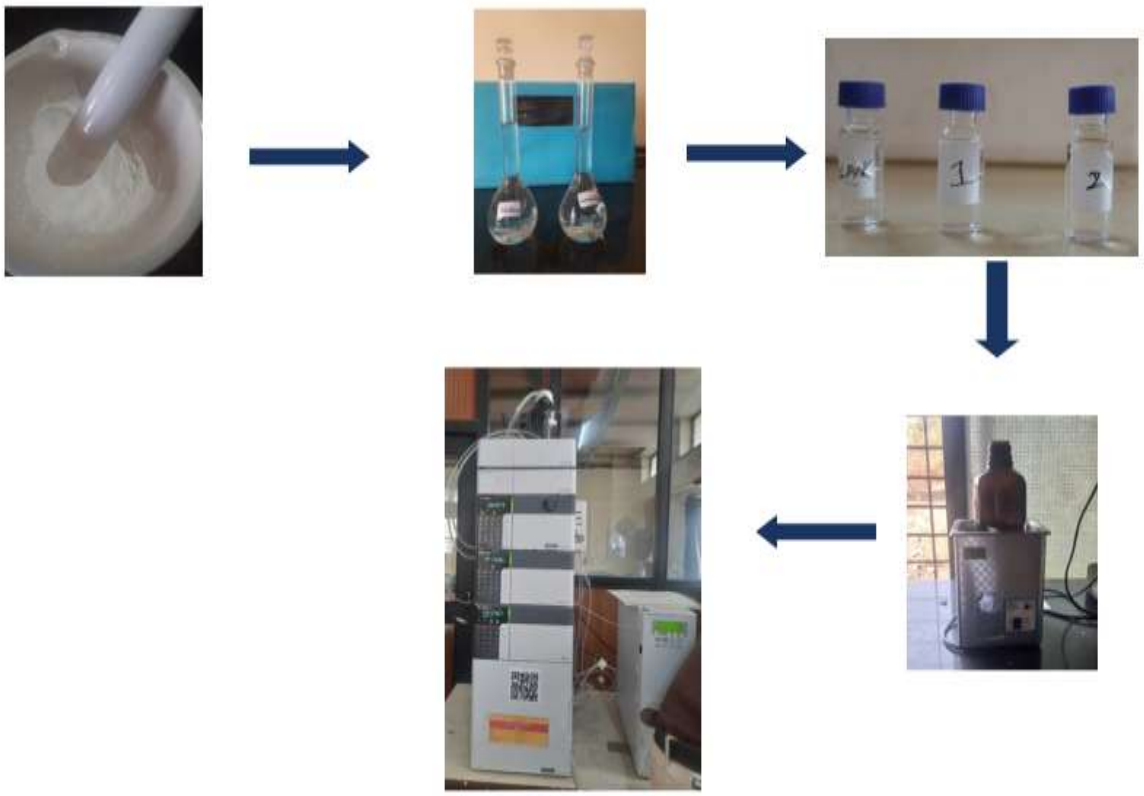

3) VALIDATION OF METHOD DEVELOPED

The method developed was validated in terms of linearity, range, accuracy, precision, the limit of detection (LOD) and limit of quantitation (LOQ), and system suitability as described in ICH guidelines Q2 (R1).

- Linearity

To confirm the linearity of the method, the calibration curve was plotted which was found to be linear over the range of 10-60 µg/ml as depicted in (Table 3) and (Fig 3). Three injections were done at each concentration. The linearity of the calibration curve was determined using the plot of peak areas vs. concentrations of the analyte. A strong correlation between the peak heights and the concentration of the drug was observed. The typical regression equation for the calibration plots was to be y = 113.75x + 762.2, with an R2 value of 0.9899, indicating a high degree of linearity. This information is also presented in (Table 2), which demonstrates that the method exhibited a linear relationship between the measured peak areas and the concentrations of Chlorpromazine.

Table 2. Selected HPLC conditions

HPLC system parameters	Conditions
Column	C18 column (250×4.6 mm, 5µm)
Mobile phase	Acetonitrile: water, pH 3.5 (40:60) (v/v) ratio
Flow rate	1.0 ml/min
Detection	260 nm
Detector	Ultraviolet (UV) detector
Temperature	25ºC
Standard volume	20 µl

Table 3. Linearity of CLN

S.No.	Conc. (µg/ml)	Area
1	10	2015
2	20	3056
3	30	4215
4	40	5862
5	50	6256
6	60	7896

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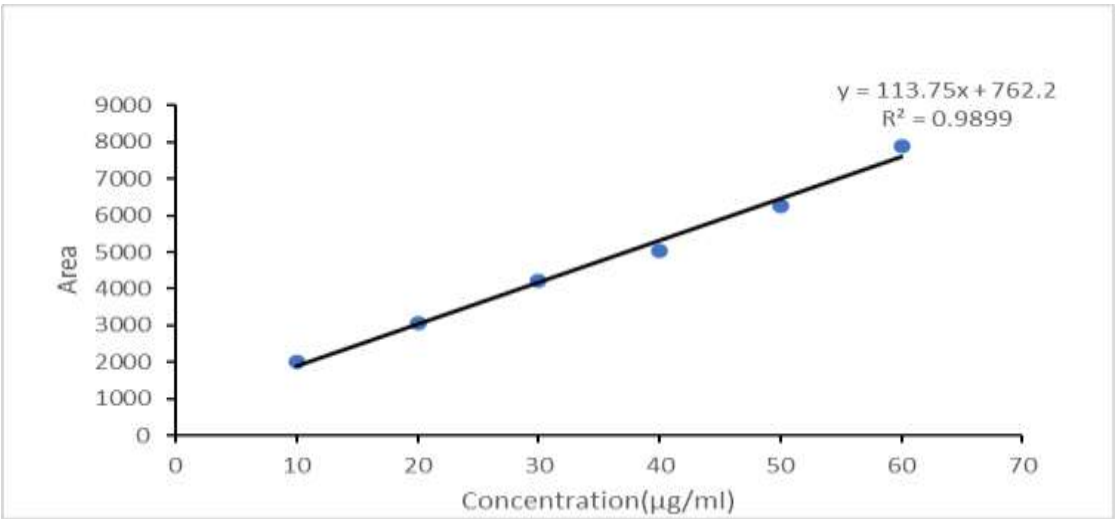

- System suitability test

According to United States Pharmacopoeia, 2007 system suitability tests are an integral part of the LC method in the course of optimizing the conditions of the proposed method [9,10]To ascertain its effectiveness, certain system suitability test parameters were checked by repetitively injecting the drugs solution at the concentration level of 10 µg/ml. Data obtained from suitability studies verified all of the parameters showed satisfactory peaks. The low %RSD of CPZ demonstrated good resolution represented in (Table 4).

Table 4. System suitability studies for developed method and values of the calculated theoretical plate, tailing factor, resolution, and retention times

Drug	Mean	%RSD	Theoretical plate	Tailing factor	Resolution	Retention Time
CPZ (20 µg/ml)	3056	0.64%	6597	0.94	4.12	3.63 ± 0. 23
USP recommendation		< 2	>2,000	0.8 to 1.5	>2	

Values were represented as their mean n =5

- Inter and intra-day precision and accuracy

Precision was determined by repeatability, interday, and intraday reproducibility experiments [11-12] as shown in (Table 4). A standard solution containing drugs of different concentrations (10, 30, and 60 mg/ml) was injected three times. For CPZ, % Relative standard deviation values were found in a range from 0.026 to 0.088 for intraday and from 0.01 to 0.09 for interday. All the values fall within the limit (limit RSD <2.0%) [22]. The produced intraday and inter-day precision revealed that the method is precise as indicated in (Table 5).

Table 5. Inter and Intra-day precision

Concentration assayed (µg/ml) (n= 3)	Intra-day (repeatability)		Inter-day (Intermediate)	
	Average area		Average area	
	Mean	%RSD	Mean	%RSD
10	2015	0.016	2101	1.011
30	4215	0.018	4025	0.126
60	7896	0.095	7789	0.246

- Accuracy/ recovery studies

If the accuracy of the developed method falls between 98 and 102% it is considered acceptable [23].

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For calculating the recovery of CPZ three different concentration levels (25, 50, and 75%) were used. As shown in (Table 6). The resulting mixtures were analyzed and the results obtained were compared with the results expected. The developed method was accurate as drug recoveries of CPZ at the selected wavelength were confirmed to be within the acceptable range of 98.2 -102.68%. Satisfactory recoveries with small percentage relative standard deviations (RSD) were obtained, indicating the method's high accuracy.

Table 6. Recovery studies of CPZ

Level Conc. %	Target conc. (µg/ml)	Total conc. (µg/ml)	Area found	Found conc. (µg/ml)	Percent recovery	Mean percent recovery	Standard deviation
25	50	12.5	2087	12.20	102.45	100.99	1.011
25	50	12.5	2088	12.14	102.96		
25	50	12.5	2190	12.81	97.58		
50	50	25	4023	24.85	101.01	101.68	0.126
50	50	25	3956	24.54	103.10		
50	50	25	4123	24.89	100.73		
75	50	37.5	5487	36.59	99.48	99.20	0.246
75	50	37.5	5490	36.66	99.09		
75	50	37.5	5493	36.67	99.03		

- Robustness

Robustness is the measure of the capacity of analytical methods to remain unaffected by small but deliberate variations of the operating conditions. A total number of five replicates were injected and %RSD was calculated. Variation of the pH of mobile phase acetonitrile: water in a ratio of 40:60 v/v with a pH of 3.5, adding glacial acetic acid by ± 2%, composition of the mobile phase by ± 2%, flow rate ± 0.1ml/min [13]. Small changes in all these parameters would not affect the method, which proves that the method was robust as shown in (Table 7.1) [24, 25].

Table 7.1. Robustness values of CPZ

Parameter (15 µg/ml solution used)	Method condition		%RSD (Peak area)	%RSD (Retention time)
Flow rate(ml/min) (±0.1)	0.9		0.94	1.50
	1.0		0.64	1.08
	1.1		1.11	1.04
Mobile phase	30:70		0.94	1.50
	40:60		0.64	1.08
ACN-Water (v/v) (±2%)	50:50		1.11	1.04
pH (±0.5%)	3.0		0.94	1.50
(±0.5%)	3.5		0.64	1.08
	4.0		1.11	1.04

- A QbD approach for determination of Robustness

During validation studies, chromatographic factors such as flow rate, mobile phase ratio, and pH were deliberately adjusted. Regardless of these changes, all outputs manifested remarkable and adequate results, followed by consistent elution. This indicates that the chromatographic conditions were optimized to obtain the desired separation of the analytes. The key factors, including peak area and retention time, remained stable even after variations in flow rate, mobile phase ratio, and pH. During the robustness studies, it was observed that the flow rate was inversely proportional to the retention time and directly proportional to the number of theoretical plates. However, the tailing factor was also found to increase, which may be due to the higher column

pressure generated by the increased flow rate. Acceptable ranges were established: flow rate ± 0.1, pH ± 0.2, and mobile phase composition ± 2%. The % RSD values obtained after these adjustments suggest that the method is robust for its intended purpose, as shown in Table 7.1 using the conventional approach.

For the novel A QbD approach, a CCD design was utilized to conduct a robustness study, as indicated in Table 7.2. To study cause and effect, the Ishikawa fishbone diagram was used to systematically identify and analyze root causes, aiding in effective problem-solving and decision-making within organizations, as illustrated in Fig 6. Critical method parameters (CMPs) or factors such as pH (A), % organic content(B), and flow rate (C) were selected at three levels. Four Critical Quality Attributes (CQAs) considered were retention time (Y1), area(Y2), tailing factor(Y3), and number of theoretical plates(Y4). The application of Analysis of Variance (ANOVA) to all response variables was used to assess the significance of the model, which showed that all responses had significant differences in their values, The equations derived from the model are as follows

Retention Time Y1 = +4.22 +0.0900A+ 0.0800B-0.2200 C+0.0000AB+0.0750AC+0.1750 BC-0.0591A^2+0.1909B^2+0.0909C^2
(3)

Area Y_2 = + 25535.00 + 138.90 A+ 736.10 B- 111.60 C-827.50 AB + 112.75 AC- 1276.75 BC (4)

Tailing Factor Y_3 = +6596.88 – 11.80A+76.40 B – 123.30C + 324.87 AB+227.62 AC-92.88 BC+433.05A2-304.95B2-293.45C2
(5)

Theoretical Plate Y_4 = +1.13 + 0.0690 A-0.0280 B - 0.0040 C +0.2525 AB + 0.2700 AC + 0.2725BC
(6)

From the equations, a positive sign indicates a positive effect and a negative sign indicates an antagonistic effect in the polynomial equation. From the table of ANOVA, responses Y_1, Y_2, Y_3, and Y_4 indicated that predicted values for all the factors are under satisfactory value. The quadratic method was suggested by software for R.T, and tailing factor whereas Area and theoretical plates were suggested (2FI) model P-values (less than 0.05) were found to be significant for all responses.

Graphical interpretation in the form, of 3-D response surface plots and contour plots, showed the correlation of the effect of factors on the response retention time, area, tailing factor, and theoretical plates of FA. The model was assessed for the effect of individual factors on the responses in the form of 3-D response surface plots and contour plots. In Design Expert software, 3D plots and contour plots serve as powerful visualization tools for analyzing experimental data and understanding the relationships between variables in a design space.

The main purpose of 3D Plot is to visualize response surfaces, which depict the relationship between multiple independent variables (factors) and a response variable (output). By plotting the response variable on the z-axis and two factors on the x and y-axis, you can see how the response changes as the factors vary, 3D plots can help identify regions of the design space where the response variable is optimized or minimized. This is particularly useful in optimization studies where the goal is to find the best combination of factors to achieve a desired outcome. It also helps in interpreting interactions between factors. Patterns in the 3D plot can indicate whether factors interact synergistically, antagonistically, or independently.

Overall, both 3D plots and contour plots in Design Expert software are essential tools for visualizing and interpreting experimental data, identifying trends, and making informed decisions in the design and optimization of processes or systems. All responses in 3D and contour plots were indicated in Fig (7 a-h), In the context of Design Expert software, perturbation generally refers to the systematic variation of factors or variables within a design matrix. This variation helps in understanding the system's sensitivity or process to changes in these factors.

Purtabation is a measure of deviation from the center point (reference) During the perturbing of a design in Design Expert, slight change in one factor, either as pH (A), % organic content(B), and flow rate (C) at a time from a reference point which is the center point of design space and simultaneously change in responses i.e Y_1,Y_2, Y_3, and Y_4 was observed Perturbation Plots gives us the effect of every factor independently when only one factor is altered and the other kept constant, which can provide information about robustness as indicated in Fig. (8 a- d). In the Perturbation plot, If the line representing a factor (A, B, C) is relatively flat, it indicates that the response variable is not significantly affected by changes in that factor. A steep slope suggests that the response variable is highly sensitive to changes in that factor, whereas If the line shows curvature, it indicates a nonlinear relationship between the factor and the response variable. Thus in the Perturbation plot of Retention time more deviation from the center point was observed and concluded that a curvature line was observed indicating a nonlinear relationship between the factor (A, B, C) and the response variable(R.T) . Similarly in Purbation plot of area less deviation of the factor was observed from the reference point indicating steep slope, which suggests that the response variable (area) is highly sensitive to changes in that factor, Probation plot of theoretical Plate indicated deep curvature hence more deviation of the factor was observed from the reference point indicating a nonlinear relationship between the factor and the response variable, However tailing Factor plot Showed very less deviation from the center point indicating flat lines, suggesting that response variable is highly sensitive to changes in that factor.

Table 7.2. Central Composite Design for robustness testing using factors and obtained responses.

Factors			Responses			
A: pH	B: % Organic	C: Flow rate	Retention Time	Area	Tailing Factor	Theoretical Plates
3	40	1	3.67	2542	0.88	6597
3.5	45	0.9	3.64	2698	0.84	6658
3	45	1	3.63	2598	0.95	6687
2.5	45	0.9	3.64	2897	0.98	6584
3	40	1	3.65	2321	0.97	6625
3	40	1	3.64	2512	0.98	6478
2.5	45	1.1	3.65	2542	0.97	5624
2.5	40	1	3.65	2561	0.87	6987
2.5	35	1.1	3.67	2512	0.99	6635
3	35	1	3.64	2542	0.97	5698
3.5	35	0.9	3.61	2489	0.94	5998
3	40	1	3.71	2687	0.98	6421
3.5	40	1	3.74	2542	2	6874
2.5	35	0.9	3.67	2256	2.1	6985
3	40	1.1	3.64	2564	0.99	5987
3	40	1	3.67	2542	0.94	6984
3.5	35	1.1	3.68	2689	0.92	6320
3	40	1	3.68	2512	1.5	6874
3	40	0.9	3.98	2568	0.95	6421
3.5	45	1.1	3.78	2489	1.9	6847

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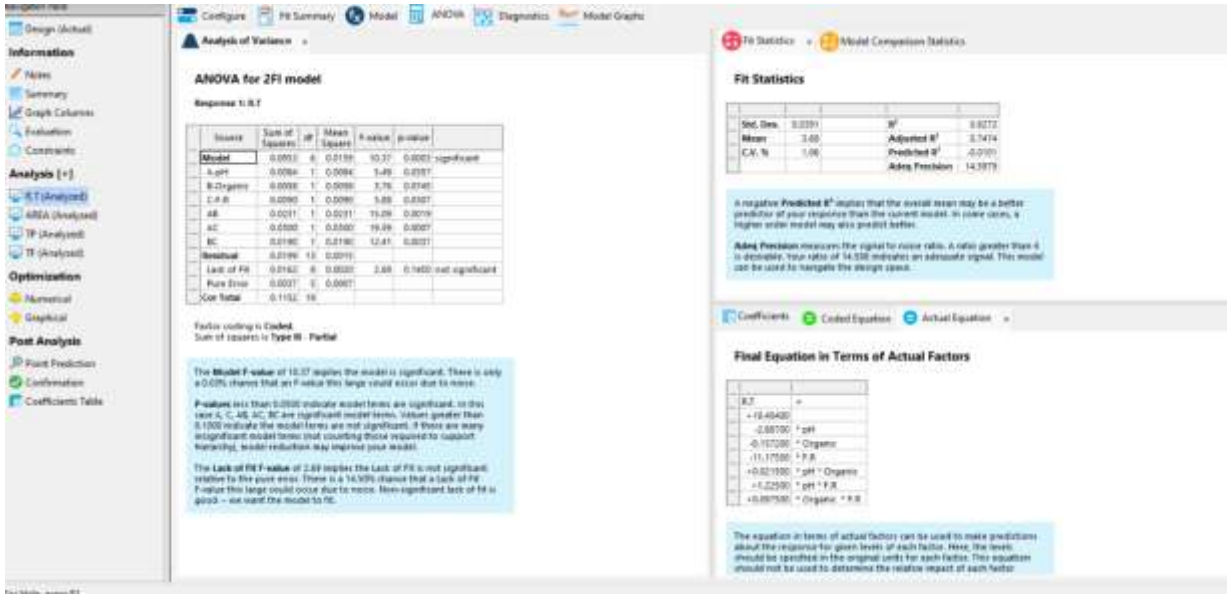
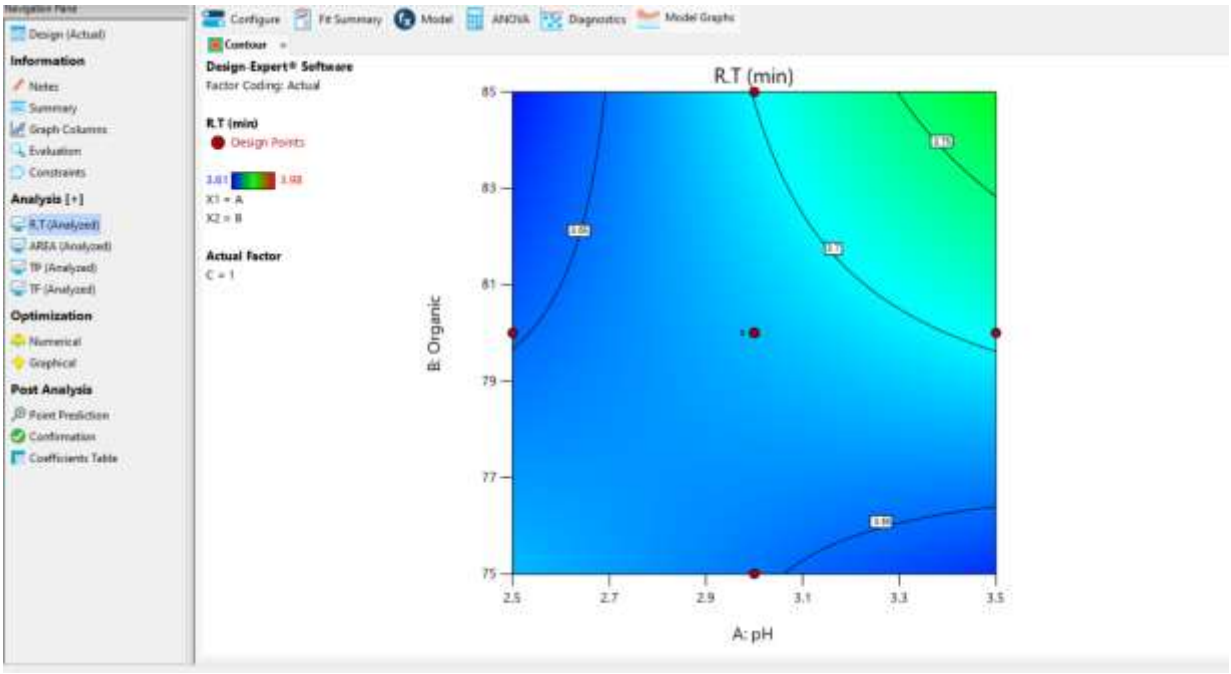
Retention time-





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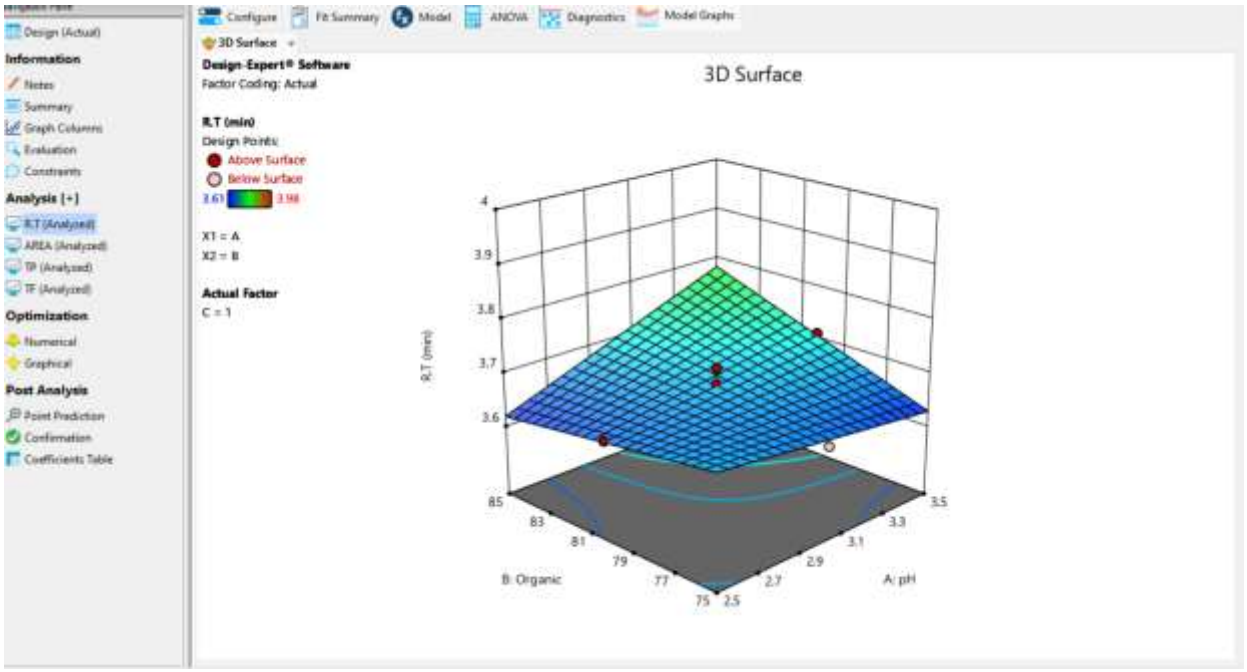
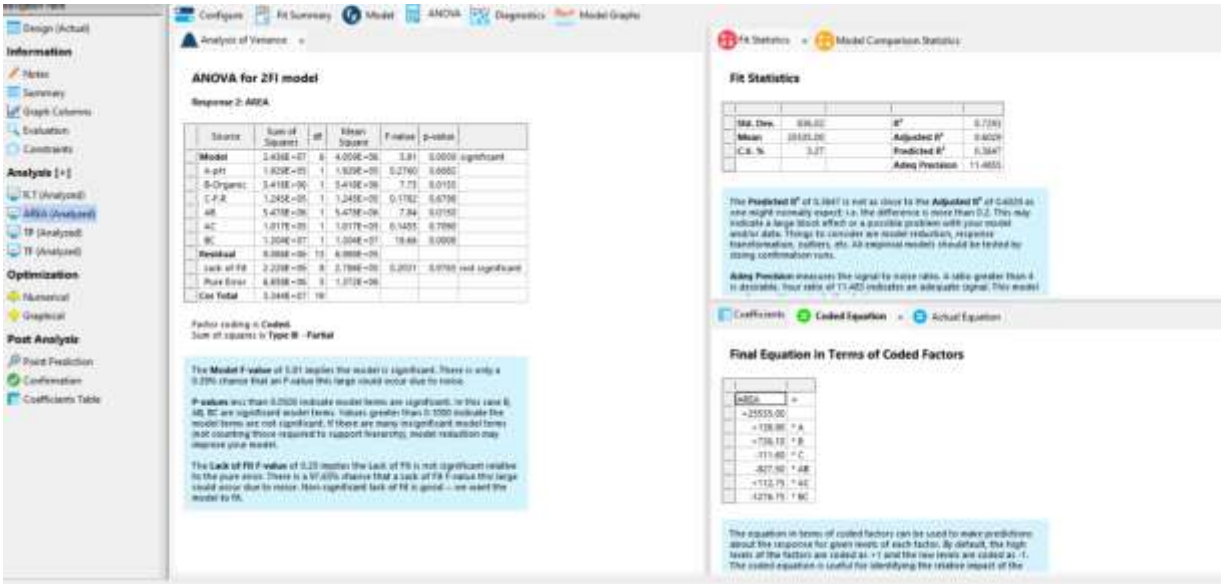


AREA



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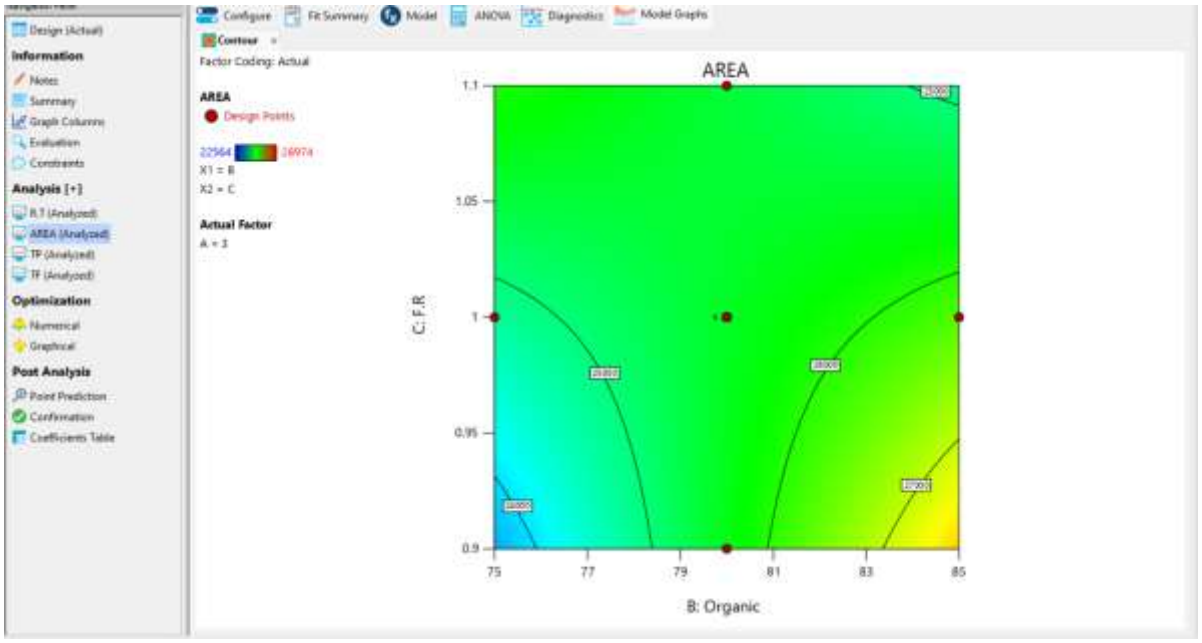

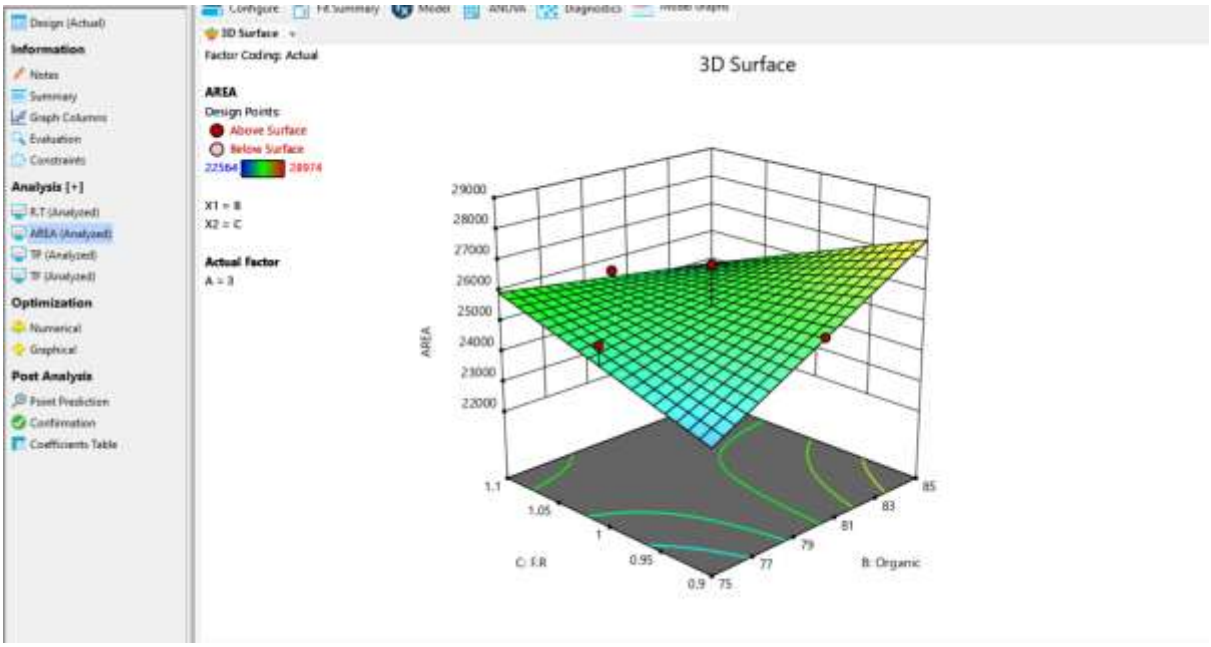

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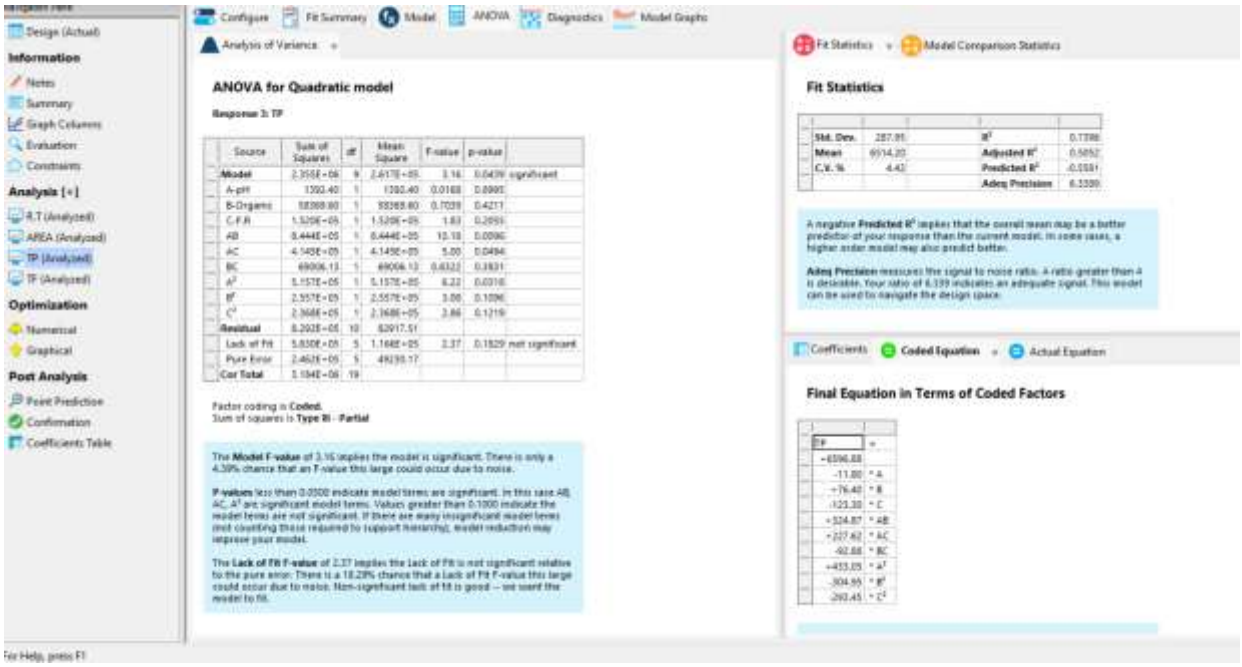
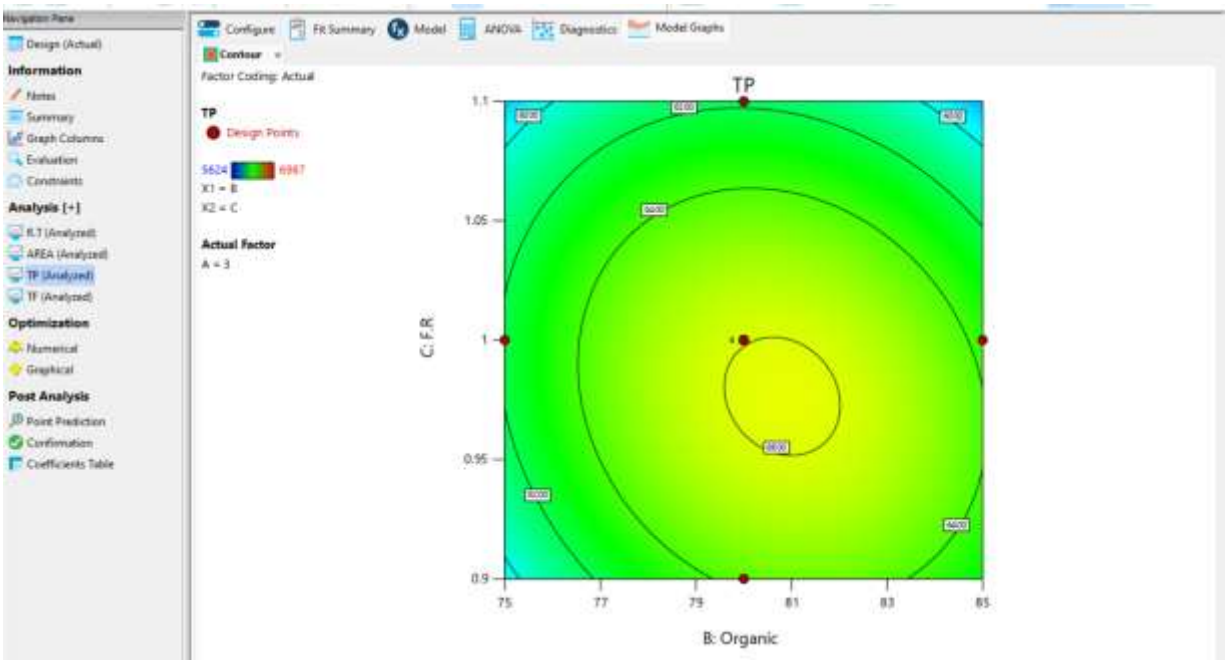
Theoretical Plate





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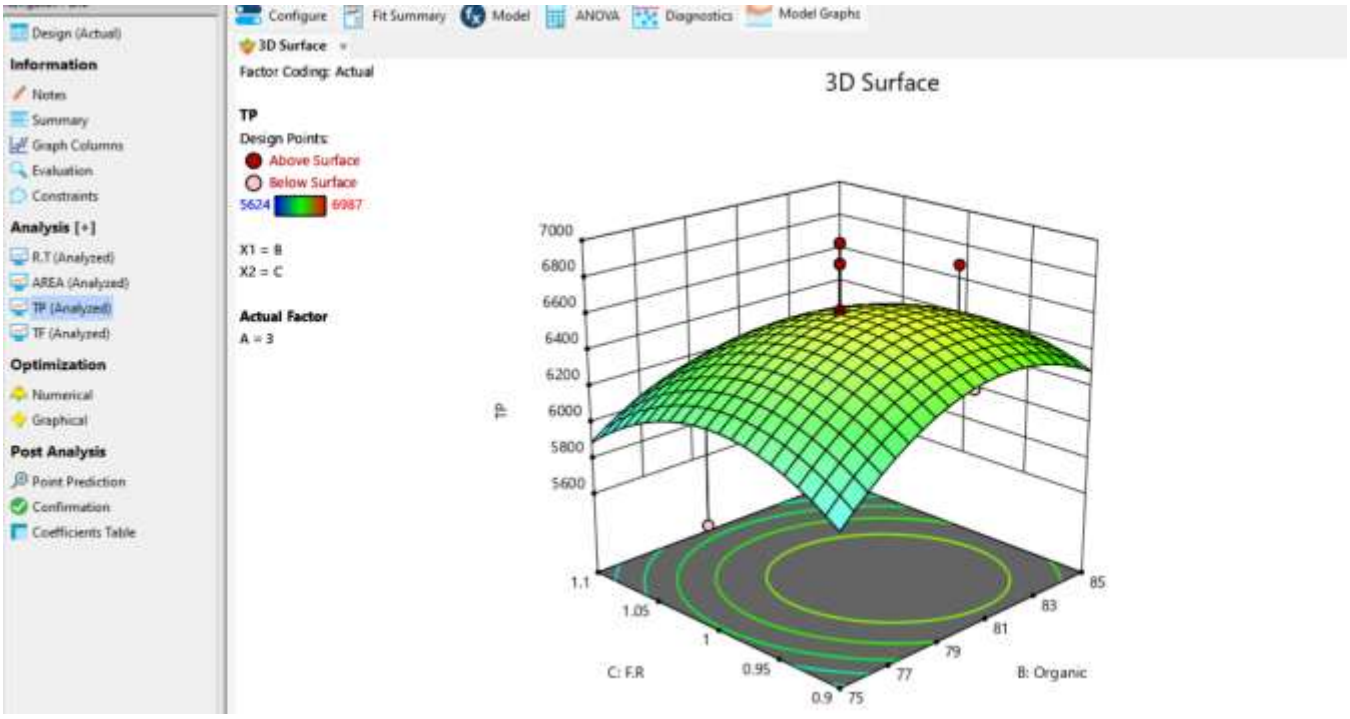
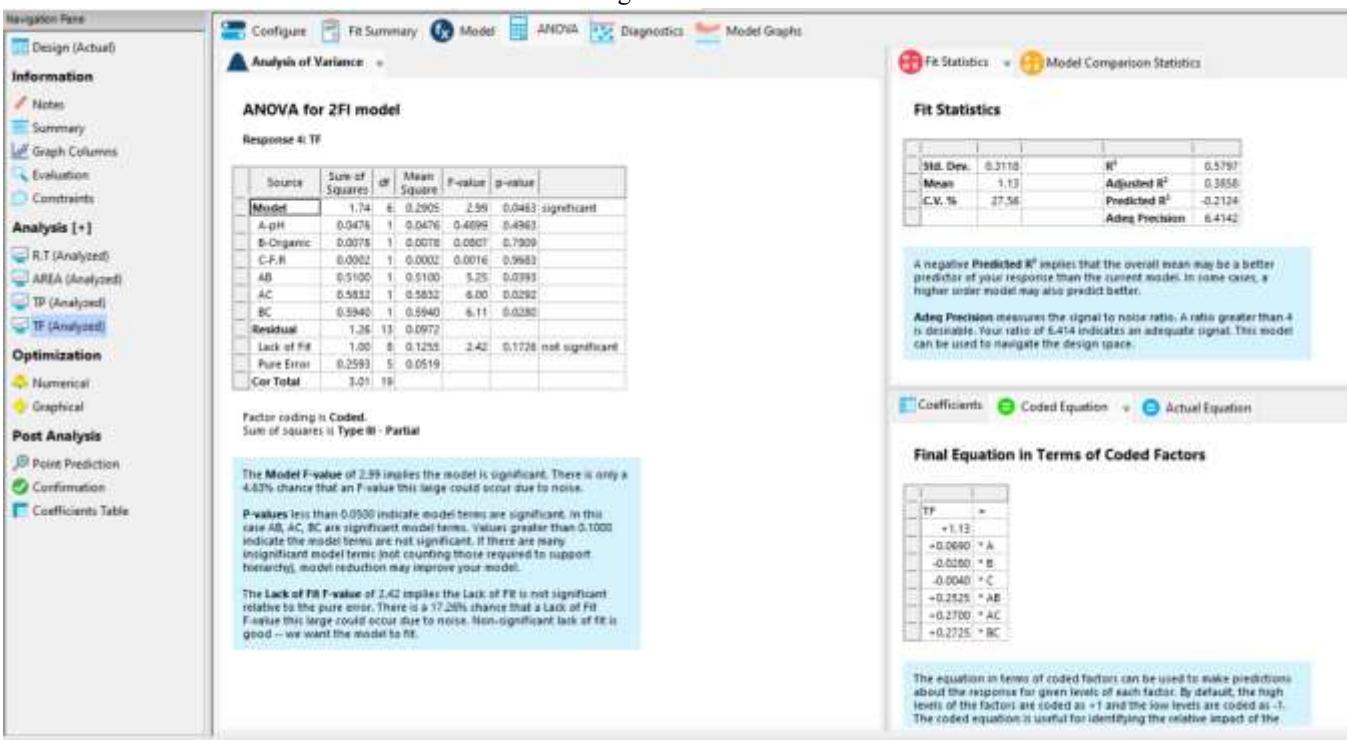


Tailing factor



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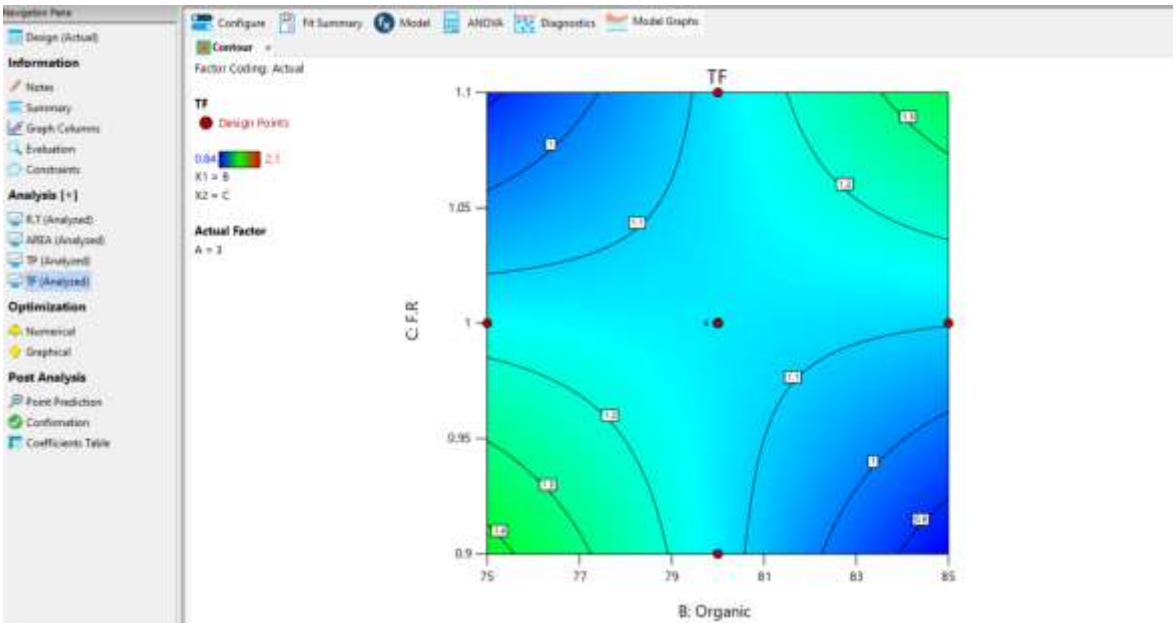

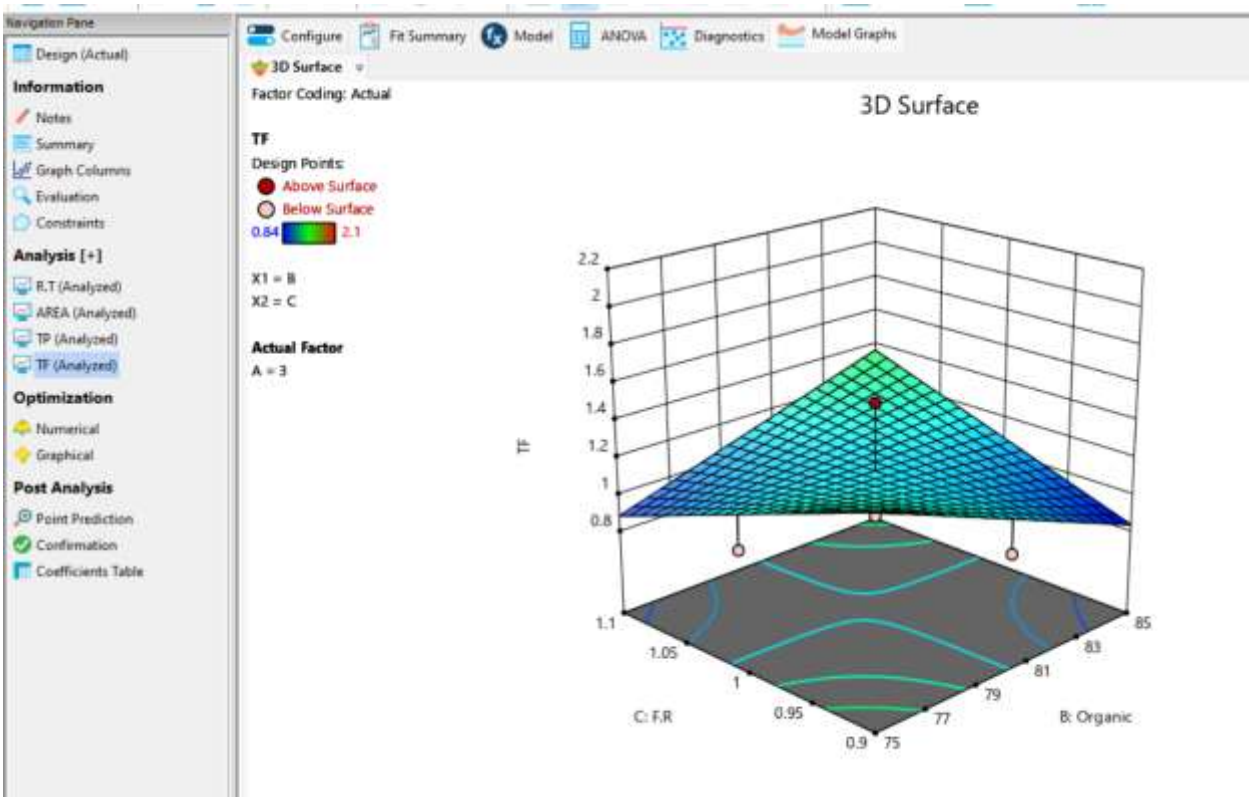

- Specificity

During, the developed method it was analyzed (Fig. 2). no interface was observed even by injecting separate injections of placebo, mobile phase, and active drugs. No additional interactive peaks were observed, and the peaks of CPZ which were observed previously at R.T 4.2 remained there and no change in retention time appeared as illustrated in (Fig. 2).

- LOD and LOQ

In the present study, the LOD and LOQ were calculated according to the 3.3 σ/s and 10 σ/s formulae, respectively; where σ is the standard deviation of the peak areas and s is the slope of the corresponding calibration curve. The LOD was 0.67 µg/ml and LOQ was 1.5 µg/ml as indicated in (Table 8) respectively [26].

Table 8. LOD and LOQ values of CLN

S.No.	Parameter	Value (µg/ml)
1.	LOD	0.3
2.	LOQ	1.1

VII. CONCLUSION

A simple, specific, accurate and reproducible high performance liquid chromatography method was developed for Chlorpromazine in it pure form. Hence, the developed HPLC method can be useful for the quantitative determination of Chlorpromazine and it's pharmaceutical formulations.

REFERENCES

[1] Walfish, S. Analytical methods: a statistical perspective on the ICH Q2A and Q2B guidelines for validation of analytical methods. Biopharm. Int. 2006, 19(12), 1–6.

[2] Shaji, J.; et, al.Development and validation of a reverse phase HPLC method for determination of meloxicam in pharmaceutical dosage forms and human plasma. Int. J. Pharm. Sci. Rev. Res. 2012, 12(1), 152–60.

[3] Qi, M.-L.; et , al. Validateda liquid cpalatography method for assay of tizanidine in drug substance and formulated products. Analytica Chim. Acta 2003, 478(2), 171–7.

[4] Fuster, J.; et, al. HPLC-UV method development and validation for the quantification of ropinirole in new PLGA multi particulate systems: microspheres and nanoparticles. Int. J. Pharm. 2015, 491(1), 310–7.

[5] Segundo, et, al.Development and validation of HPLC method with fluorometric detection for quantification of bisnaphthalimidopropyldiaminooctane in animal tissues following administration in polymeric nanoparticles. J. Pharm. Biomed. Anal. 2016, 120, 290–6.

[6] Pethkar, S. Development and validation of UV spectrophotometric method for analysis of nortriptyline hydrochloride in bulk and tablet dosage form. World J. Pharm. Pharm. Sci. 2016, 5(7), 1452–9. 46.

[7] S.; Özaydin, T et, al. Development and validation of a reversed-phase HPLC method for the determination of lisinopril and gliclazide in pharmaceuticals. Marmara Pharm. J. 2017, 21(2), 338–44. 47.

[8] Akhlaq, M, et, al. Abdelkader, H. A simple high-performance liquid chromatographic practical approach for determination of flurbiprofen. J. Adv. Pharm. Technol. Res. 2011, 2(3), 151.

[9] International Conference on Harmonisation. 1994. Test on Validation of Analytical Procedures, US FDA Federal register.

[10] Lu, C.; et, al.Simultaneous determination of ivabradine and N-desmethyl ivabradine in human plasma and urine using an LC-MS/MS method: application to a pharmacokinetic study. Acta Pharm. Sinica B 2012, 2(2), 205–12.

[11] Vella Szijj, et, al. The combined effects of pH and acetonitrile composition on the separation of two Lincosamide antibiotics. Asian J. Pharm. Clin. Res. 2014.

[12] Moldoveanu, et, al. Parameters that characterize HPLC analysis. Essentials Mod. HPLC Separations 2013, 53–83.

[13] Moldoveanu, S.C.; et, al. Parameters that characterize HPLC analysis. Essentials Mod. HPLC Separations 2013, 53–83.

[14] Hasan, N.; et, al.Dual wavelength RP-HPLC method for simultaneous determination of two antispasmodic drugs: an application in pharmaceutical and human serum. J. Anal. Methods Chem. 2013, 20.

[15] Chandraiah, M.R.; et, al. RP-HPLC method for estimation of itopride hydrochloride from the tablets dosage form. J. Chem. Pharm. Res. 2012, 4(5), 2649–51.

[16] Suneetha, D.; et, al.RP-HPLC method for the estimation of eletriptan in pharmaceutical dosage forms. Int. J. Chem. Environ. Pharm. Res. 2010, 1(2), 95–9.

[17] Kaul, N.; et, al.Application of HPLC and HPTLC for the simultaneous determination of tizanidine and rofecoxib in the pharmaceutical dosage form. J. Pharm. Biomed. Anal. 2005, 37(1), 27–38

[18] Rajan, V.T.; et, al. A simple RP-HPLC method for quantitation of itopride HCl in the tablet dosage form. J. Young Pharm. 2010, 2(4), 410–3.

[19] Perumal, S.S.; et, al. Analytical method development and validation of simultaneous estimation of rabeprazole, pantoprazole, and itopride by reverse-phase high-performance liquid chromatography. J. Food Drug Anal. 2014, 22(4), 520–6.

[20] Naik, D.S.; et, al.Method development and validation of eletriptan hydrobromide pharmaceutical dosage form by RP-HPLC. Int. J. Eng. Res. Appl. 2013, 3(6), 1–5

[21] Shao, Y.; et, al.A stability indicating HPLC method for the determination of glucosamine in pharmaceutical formulations. J. Pharm. Biomed. Anal. 2004, 35(3), 625–31.