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Method Development and Validation of Clozapine by HPLC-An QbD Approach

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[2]Method Development and Validation of Clozapine by RP-HPLC - an QbD Approach

ABSTRACT

Background: Quality by design, or QbD, is the process of achieving a planned, predictable quality with intended and predetermined requirements. A key component of QbD is comprehending dependent variables, various factors, and their interaction effects by a desired set of trials on the response to be researched. The current study describes the development of a risk-based HPLC approach, its validation, and stability studies for Clozapine in pharmaceutical dosage form.

Results: The mobile phase and flowrate, two crucial components of the RP-HPLC process, are the key components of an efficient experimental design that is discussed. The chromatographic conditions were optimized using Design Expert* (Version 12.0.3. PC Wonderland - Stat - Ease - Design - Expert -12x64), column c18 (250mm*4.6mm, 5.0), methanol as the mobile phase, 1 ml/min flow rate, and retention time of 2.007 min. The developed method was found to be linear at a detection wavelength of 260 nm, with a r2 value of 0.9998 for the range of 2-12 g/ml. The parameters for the system suitability test, theoretical plate, and tailing factor were found to be 1.369 and 38672, respectively. RSD percentages for intraday and inter day precision were found to be 0.0361 and 0.0360, respectively. The validation of the method

CONCLUSION: The central composite design experimental design, which depicts the interactions of mobile phase and flowrate at two different levels, revealed the tailing factor and retention time with the design expert 12.0 version. Here, the factors influencing chromatographic separation are better known, and it is more certain that the developed HPLC process will be able to accomplish its objectives. The test was carried out. There are no peaks that coelute with the clozapine peak, according to the data on chromatographic peak purity. The QbD methodology was applied at various phases to build analytical methods and enhance knowledge of method variables.

Keywords: Clozapine, stability studies, quality by design, and HPLC.

Background

A QbD is defined as a systemic approach to the method that begins with the establishment of clear goals and places an emphasis on the product as well as the understanding of the standards and processing of the product controls. The QbD's agenda includes comprehending and putting into practice ICH Q8 pharmaceutical development standards. The atypical antipsychotic clozapine treats schizophrenia (a chronic, severe mental illness) by blocking the neurotransmitter dopamine as well as targeting the neurotransmitter serotonin. It works by blocking 5-HT2A/5-HT2c serotonin receptors and D1-4 dopamine receptors.

The current endeavor seeks to develop and improve the RP-HPLC method for Clozapine in pharmaceutical dosage utilizing a quality-by-design methodology.

METHODS

Materials

The clozapine was received as a free sample from Sun Pharmaceuticals Pvt. Ltd. in Vadodara, India. Solvents and all other chemicals were of analytical grade. The experiment made use of the 50mg dosage of sizopin that is available for purchase.

Instruments and reference standards

The HPLC WATERS -2695 was used at room temperature with the C18 column (150mm*4.6mm*5m particle size), along with the UV VIS Dual Absorbance Detector WATERS-2487.

Chromatographical conditions

The Phenomenex C - 18 column (equilibrated with 100% methanol mobile phase, 250 mm*4.6 mm, 5.0 m particle size) was used. The flow rate was held constant at 1 ml/min, and the column's temperature was set to ambient. At 260 nm, eluents were observed using a PDA detector. The drug was separated with a suitable degree of peak symmetry under the aforementioned chromatographic conditions. The mobile phase and flow rate were optimized using the central composite design as two variables at two separate levels for the HPLC procedure for clozapine.

preparation of a standard reference solution

To make the 100g/ml standard stock solution, 5mg of exact clozapine was dissolved in 50ml of methanol. The stock solution was further diluted to 10 g/ml to create the sub stock. A 2g/ml solution was made by adding methanol to 2ml of sub stock solution.

Selection of detection wavelength

260 nm was chosen as the detection wavelength for 2ug/ml of clozapine after it was scanned between 200 and 400 nm.

HPLC method development using the QbD strategy

HPLC method developed by QbD in accordance with.

A quality target product profile (QTPP)

The QTPP is essential for determining the variables that affect other variables, such as theoretical plate and peak tailing, in the proposed HPLC method.

Determine the technique parameter that the critical quality attributes (CQA)

CQA has a direct impact. The mobile phase's composition and flow rate in millilitres per minute must remain constant throughout the QTPP's permitted response range.

Factorial design

The mobile phase and flow rate of the HPLC procedure were optimized and selected utilizing the central composite experimental design after the QTPP and CQAs had been established. The different interactions quadratic influences of the mobile phase composition and flowrate ml/min on the fictitious plate and peak tailing were examined using a central composite response surface randomised design.

The design was made using Design Expert* (Version 12.0.3. pc wonderland - Stat - Ease - Design - Expert -12x64) at two different settings for the mobile phase composition and flow rate. $Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_{12} AB + \beta_{11} A_2 + \beta_{22} B_2 B + \beta_{22} B_2 A + \beta_{11} A_2$

The parameters of the process and the interaction of many variables have been investigated; therefore, the factors were selected based on preliminary research. Mobile phase composition and flowrate are listed in Table 1 as independent variables, while theoretical plate peak tailing is listed as a dependent variable.

Evaluation of experimental results and selection of final method conditions

For those examined settings for theoretical plates, the procedure conditions were first tested using the CCD design. As a result, peak tailing for clozapine were assessed, resulting to distinct chromatographic conditions. Where the deliberate deviations don't impair the deemed acceptable quality ranges from the reliable regions. The method has been declared to have passed validation testing without subsequently failing. If the modelling experiment does not produce the desired outcome, try again to do so. The ideal chromatographic conditions will be optimized by utilizing the Design experiment tool.

Table 1 coded values for independent variables

Factor	**Coded values for given factor**	**Levels**		
		-1	**0**	**+1**
Methanol	A	70	100	100
Flow rate	B	0.9	1	1.1

Risk evaluation

The optimized final technique is used throughout the duration of the product's life. The robustness and ruggedness were evaluated utilizing a range of laboratories, chemicals, analyser’s, instruments, reagents, and days.

Implement a control strategy.

After the approach has been developed, a control strategy should be put into place. The analytical target profile (ATP) was created in order to create the analytical control plan, which includes fitness of purpose and analytical technique. This ensures the accuracy of the data from the ATP method's sample preparation, measurement, and replication control methods.

Improvement over time for managing the analytical life cycle

The optimal approach is to continuously check the quality attribute, and HPLC equipment, computers, software updates, and other pertinent tools and apparatus can be used for laboratory maintenance.

Validation of analytical methods

"Method validation" is the use of a certain methodology procedure to adapt the analytical process to the desired objective. Clozapine estimation using the developed HPLC was authorized in compliance with ICH Q2 (R1) criteria.

Linearity

For the linearity, six concentrations between 2 and 12 g/ml were made. To produce the calibration curve, peak area was plotted against concentration on the y axis. Calculations were made to determine values for the regression line equation and correlation coefficient**.**

Precision

For the purposes of determining intraday precision and interday precision, six samples were injected into the column on the same day and twice back-to-back days, respectively. It was calculated that the % RSD should be under 2.Table 2: Parameter optimization for clozapine CCD analysis

Run	Mobile phase	Flow rate	Theoretical plate	Peak tailing
1	70	0.9	37681	1.689
2	100	0.9	37854	1.498
3	100	1	38672	1.369
4	85	0.9	37955	1.688
5	100	1	38672	1.369
6	70	1.1	37955	1.684
7	85	0.9	38001	1.672
8	100	1	38672	1.369
9	100	1	38672	1.369
10	85	1.1	38684	1.823
11	100	1	38672	1.369
12	100	1	38672	1.369
13	85	1	38656	1.634

Accuracy

The able to evaluate the accuracy of the procedure by calculating the recovery studies from commercial formulation at three levels of 80%, 100%, and 120% of standard addition. How effectively clozapine recovered was determined. The permissible range for percentage recovery was 98-102% of standard addition, as per ICH standards.

LOQ and LOD

The LOD, which is the lowest drug concentration that can be precisely identified and distinguished from LOQ, which can be quantified at the lowest concentration, was calculated using formulas based on the standard deviation of the y-intercept of regression lines and the slope of the calibration curve.

$LOD = 3.3* \sigma/S$ $LOQ = 10*\sigma/S$

Where S is the curve's slope and is the standard deviation.

Table 3 Obtained for optimized formulation

Code	Mobile phase	Flow rate	Theoretical plate	Peak tailing
C11	100	1	38672	1.369

Robustness

By altering the pump flow rate and the number of columns, the robustness strategy was put to the test. The calculated percent RSD of the peak area acceptance limit was less than that found in two system suitability analyses.

System suitability testing was performed to evaluate characteristics including% RSD of peak area, theoretical plates, and tailing factor by injecting a working standard solution into the column.

Assay

Five tablets' weight and powder were completed. Precisely weigh 5 mg of clozapine before transferring it to a 50 ml volumetric flask filled with methanol. After that, the powder needs to be sonicated for 20 minutes to dissolve. The solution should be filtered using 0.42 Whatman filter paper. Put 1 ml of the filtrate (10 g/ml) in a flask with a capacity of 10 ml. The solution was analysed using HPLC, and the findings were calculated using the formula

%Assay = Sample area/Standard area.

STUDIES ON FORCED DEGRADATION

The forced degradation experiments were performed in accordance with ICH Q1A (R2) under a variety of stress conditions, including hydrolytic (acidic, basic), oxidative, thermal, and photolytic.

Acid degradation

0.1M HCL was employed in the investigation on acid degradation. A 10 ml flask was used to collect clozapine samples. This 10ml flask contained 0.1M HCL, which was then put into it, sonicated for 10 minutes, then refluxed at 60C for 6 hours. After being refrigerated and neutralized with 0.1M NaOH, it was diluted to 10 g/ml. The HPLC was used to analyze the acid degradation of the sample.

Base degradation

0.1M NaOH is employed. A 10 ml flask containing clozapine was used. This mixture was sonicated for 10 minutes, combined with 10ml of 0.1M NaOH, and then refluxed at 60C for six hours. After being refrigerated and neutralized with 0.1M HCL, the solution was diluted. The sample was then administered to the HPLC for analysis.

Oxidative degradation

This 30% H2O2 was used to conduct this study. 10ml of clozapine was diluted with 30% H2O2, sonicated for 10 minutes, and then refluxed at 60°C for six hours. After cooling, it was diluted to 10 g/ml. The material was put into the HPLC to be examined.

Thermal degradation

This was completed in a hot air oven at 110C. Clozapine was stored in a glass petri dish at 100C for one day. After the designated amount of time had passed, the sample was refrigerated, placed in a 10ml flask, dissolved in the solvent, and diluted to 10g/ml. The substance was placed in the HPLC for analysis.

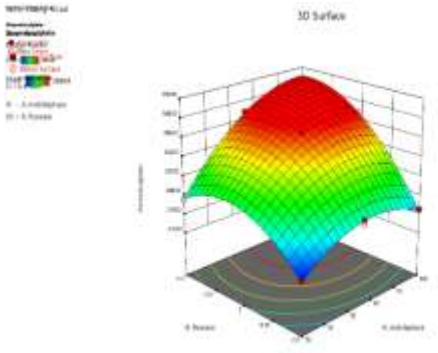
Photolytic degradation,

A sample of the drug clozapine was put in a glass petri dish and exposed to UV radiation for 24 hours. The drug was then transferred to a 10 ml flask, dissolved in the mobile phase, and the sample was diluted to a concentration of 10 g/ml after the stress. HPLC was used to prepare and analyse the material.

Results

The peak broadened when acetonitrile to water in the mobile phase was first attempted. Acetonitrile and water later failed to peak, while methanol and water did, with a peak tailing and varying flowrate. The system suitability test parameters were met with better chromatographic conditions, and the central design was then applied.

Fig. 1 shows a 3D surface plot of a combination of factors on R1 theoretical plates of clozapine by using central composite design



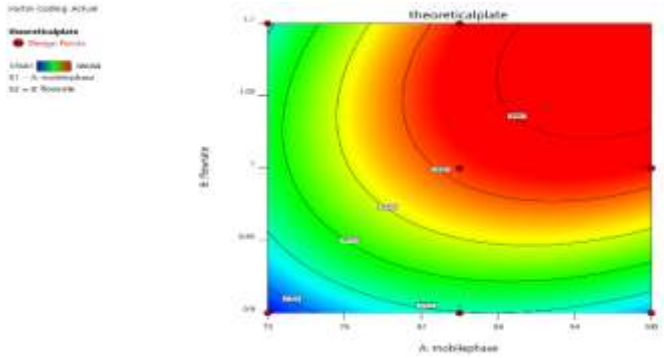


Fig 2 contour surface plot for effect of combination of factors on R2 tailing factor Clozapine by using central composite

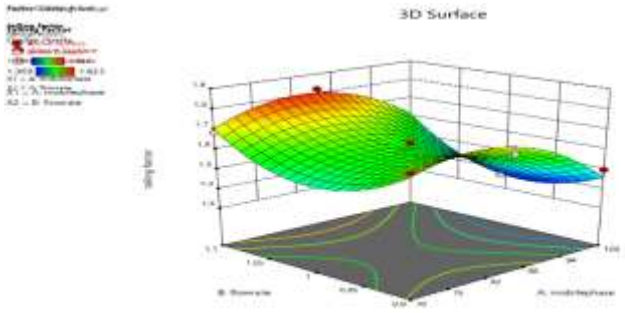


Fig 3 3D surface plot for effect of combination of factors on R2 tailing factor Clozapine by using central composite design

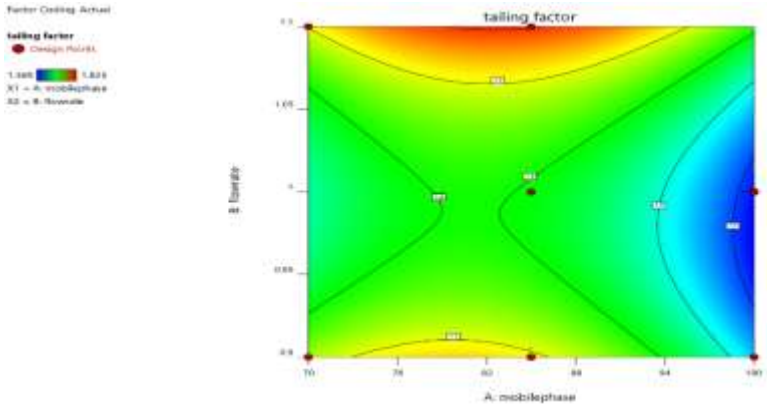


Fig 4 Fig contour surface plot for effect of combination of factors on R2 tailing factor Clozapine by using central composite

Table 4 Linearity of Clozapine

Linearity	Peak area
2	260885
4	300696
6	342344
8	379946
10	428288
12	458500

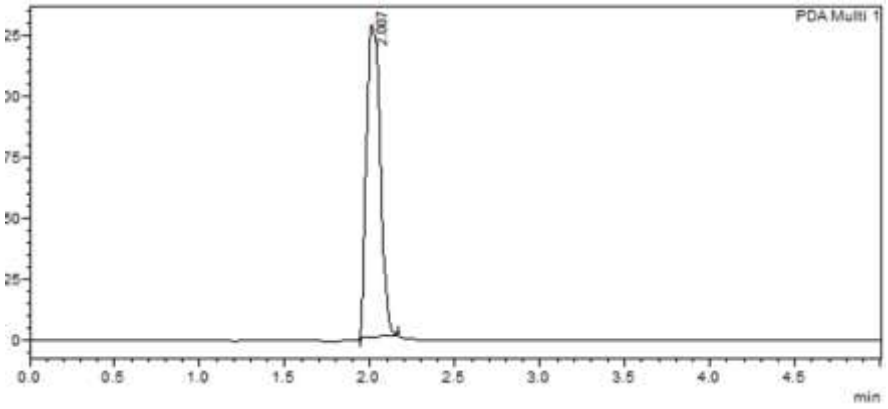


Fig 5 Linearity of 2µg/ml Clozapine

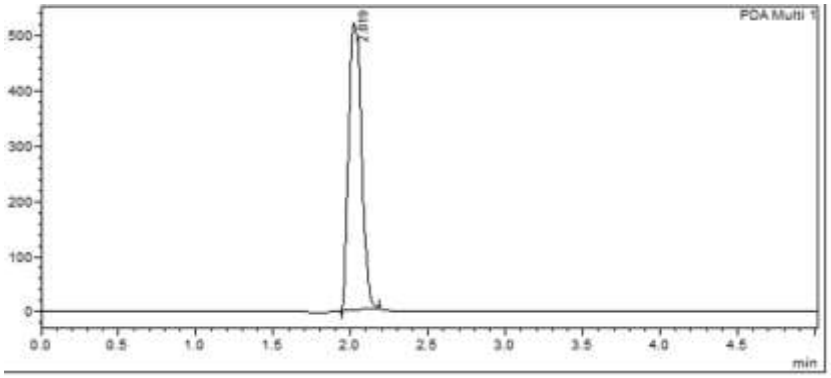


Fig 6 Linearity of 4µg/ml Clozapine

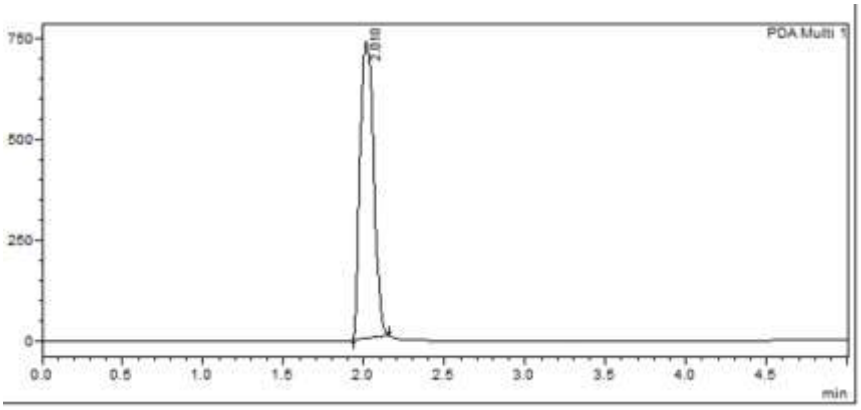


Fig 7 Linearity of 6µg/ml Clozapine

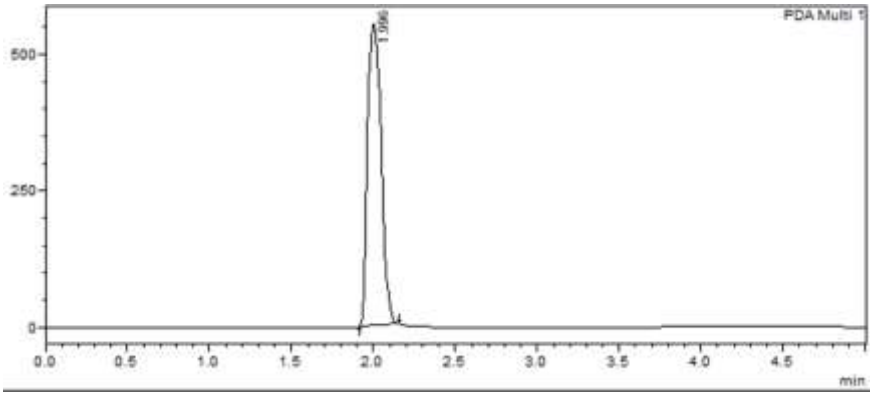


Fig 8 Linearity of 8µg/ml Clozapine

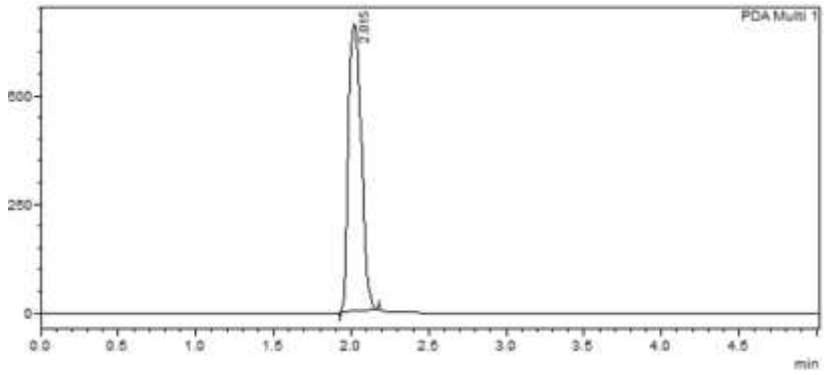


Fig 9 Linearity of 10µg/ml Clozapine

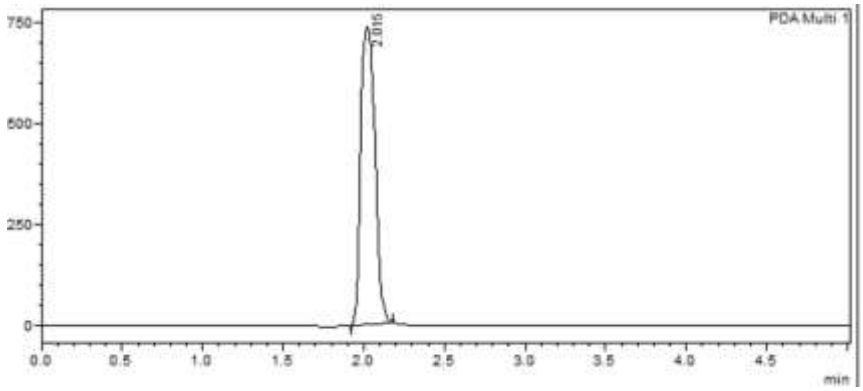


Fig 10 Linearity of 12µg/ml of Clozapine

Table 5 Data for intraday and interday of Clozapine

Concentration	Intraday precision	Interday precision
6	365720	365720
6	365942	365948
6	365891	365901
6	366081	366081
6	365972	365970
Average	365921.2	365924
Standard deviation	132.2335	131.801
RSD	0.036137	0.036019

Table 6 Recovery of Clozapine

Assay level	Amount equivalent to tablet powder	Standard added	Amount recovery	% recovery
80%	6	4.8	80%	100%
100%	6	6	101%	101%
120%	6	7.2	119%	99%

HPLC method development using the QbD strategy

Quality target product profile

The QTPPs were selected based on the projected plate and peak tailing under HPLC-optimized chromatographic conditions.

Critical quality attributes

The mobile phase is methanol, and the flowrate is 1 ml/min.

Factorial design

The CCD central composite design was chosen with the intention of developing HPLC. The optimization of numerous parameters is shown in Table 2.

Design space

We used a central composite design, a quadratic design with 13 runs, and a response surface research type. Mobile phase, flowrate, and findings from the suggested CCD experimental setup were compared to a potential plate peak symmetry.

Retention time from the equation in Fig. 1: 3867.20+276.64*A+341.25*B+194.85*AB-238.71*A2-303.85*B

The equation in Fig. 2 provides peak symmetry: +1.60-0.0749*A+0.0472*B+0.0303*AB-0.1448*A2+0.1612*B2.

Optimized condition obtained

It was created by analysing every response, and values for the responses were calculated using an HPLC chromatogram for a particular combination of mobile phase and flow rate. To determine the % prediction error, the calculated values were next compared to the projected values.

VALIDATION OF METHOD

When the system suitability test was used, the chromatogram to test the hypothetical plate was 38672, the peak symmetry was 1.369, and the RSD was 0.36. The 3D surface plot of attractiveness for formulating is shown in Fig. 4.

Linearity

The calibration curve for clozapine was linear over the 2-12 g/ml range, as shown in Fig. 2 and Table 4. The regression equation was found to be y = 19696x+222245 with a 0.9998 correlation coefficient when a graph of peak area vs concentration was constructed (Figs. 5, 6, 7, 8, 9, 10).

Precision

The inter day and intraday precisions were shown in Table 5. When the percentage RSD value was less than 2, the suggested method was shown to be accurate.

Accuracy

The accuracy was calculated as 80%, 100%, and 120% for the sample solutions. Table 6 displays the HPLC statistics for percent recovery. A recovery percentage of 98-102% supports the developed method's accuracy in compliance with ICH Q2 (R1) requirements.

Robustness

Robustness was examined with slight flowrate variations.

LOD and LOQ

The LOD and LOQ for clozapine were determined to be 0.02 g/ml and 0.06 g/ml, respectively, based on the standard deviation of the slope and intercept.

Assay

It was shown that the peak for clozapine was retention time. The medicine contained the claimed amount of clozapine, according to the assay. The results demonstrated that even when excipients were present in the tablet powder, the approach could test accurately and in particular.

FORCED DEGRADATION STUDIES

Table 6 (Figs. 11, 12, 13, and 14) shows the peak area and retention time from investigations on acidic, basic, oxidation, and heat deterioration.

The formula was used to determine the deterioration peaks.

= (% assay of stressed sample + % impurities) * 100 / % assay of unstressed sample.

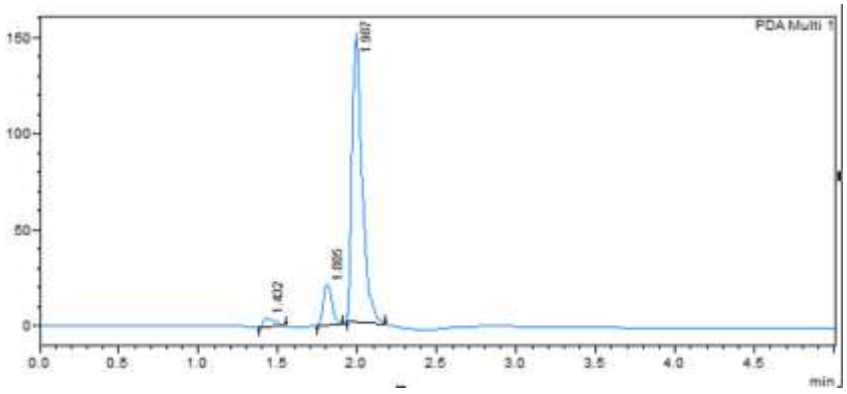


Figure 11 Chromatogram of Base degradation

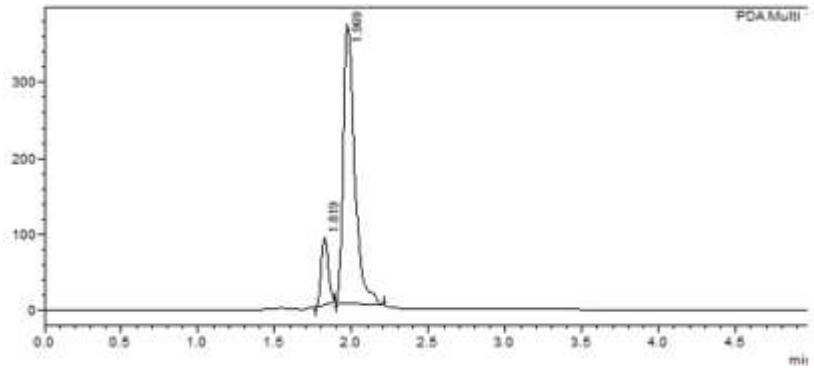


Fig 12 Chromatogram of Acid degradation

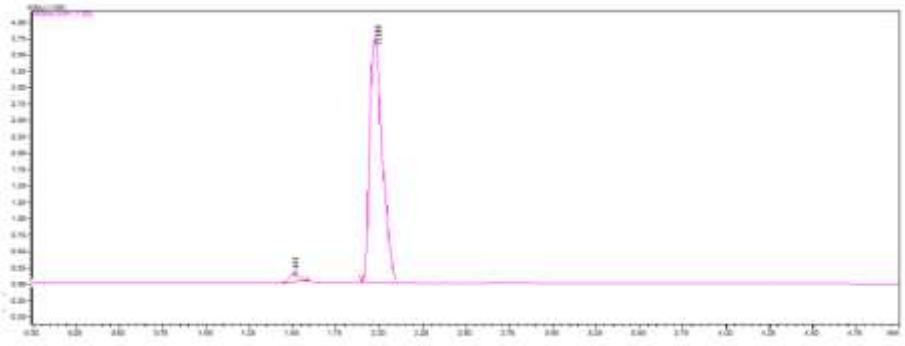


Fig 13 Chromatogram of Oxidative degradation

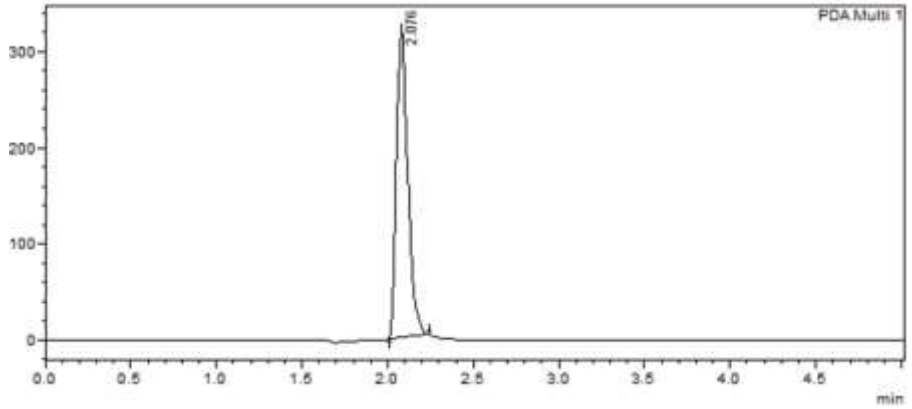


Fig 14 Chromatogram of photo degradation

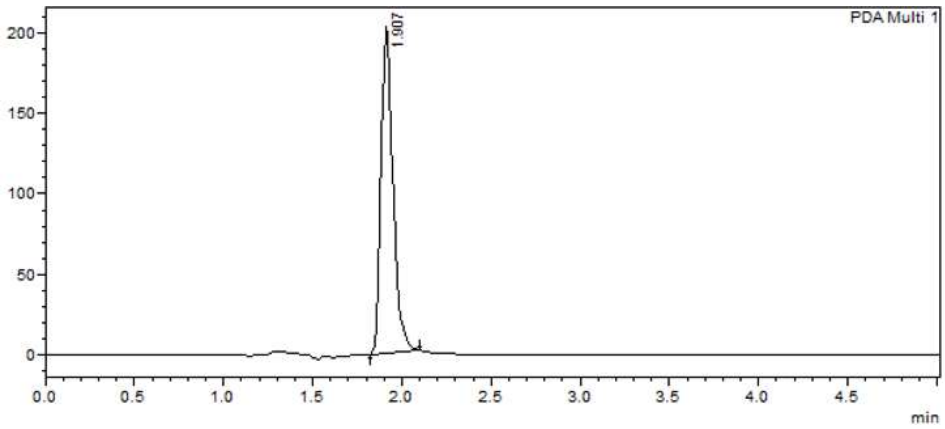


Fig 15 Chromatogram of Thermal degradation

Summary

S. No	Parameter	Clozapine
1	Linearity range	2 – 12
2	Regression equation	19696x+222245
3	Correlation coefficient	0.9998
4	Accuracy	99-101
5	Interday precision Intra day precision	0.036137 0.036019
6	LOD	0.02
7	LOQ	0.06

Conclusion

It has been described how to build an HPLC method utilizing the quality by design approach. The aims were made obvious by employing an analytical target product profile.

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Dr. B. Vasudha Bakshi Dean Department of Pharmacy, School of Pharmacy, Anurag university (CVSR) Hyderabad

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