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RP-HPLC Analytical Method of Metoclopramide Hydrochloride Prepared Nanoparticle formulation in Solid Oral Formulations by using Box-Behnken Design

Pooja Ganeshdas Bhutada[*1], Sowmya Kulal[1], Shabana Sultana[2], Mahesh Pawar[3], Parag Arun Kulkarni[4]

[1*]Senior Scientist, Formulations, Alcami Corporation.

Email ID: sowmya.Kulal@Alcami.com

[2]Assistant Professor, Department of Pharmaceutical Analysis and Quality Assurance, Shadan Women's College of Pharmacy, Hyderabad, India,

Email ID: sshab88@gmail.com

[3]Professor and Principal, KVPS Maharani Ahilyabai Holkar College of Pharmacy, Shirpur, Maharashtra, India,

Email ID: maheson@rediffmail.com

[4]Professor, Department of Pharmaceutics, Shastry Institute of Pharmacy, Erandol, Maharashtra, India,

Email ID: kparag4@yahoo.com

***Corresponding Author:**

Pooja Ganeshdas Bhutada,

Assistant Professor, Oxford College of Pharmacy, Bangalore, India.

Email ID: bhutada1994@gmail.com

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ABSTRACT

An RP-HPLC method was developed and validated for the quantitative estimation of Metoclopramide Hydrochloride from both tablet dosage forms and optimized nanoparticle formulations. Chromatographic separation was achieved on a C18 column using a mobile phase of potassium dihydrogen phosphate buffer and methanol (60:40, v/v), with detection at 273 nm. The retention time for Metoclopramide Hydrochloride was found to be 6.78 minutes. The method was validated in accordance with ICH Q2 (R1) guidelines for specificity, accuracy, precision, linearity, robustness, and sensitivity. It exhibited excellent linearity across a wide concentration range, high recovery values, and low relative standard deviation, confirming reproducibility and reliability. In the nanoparticle formulation study, the optimized batch was selected based on a high desirability value of 0.9505, achieved at guar gum 2.99%, sodium alginate 2%, and chitosan 1.23%. The formulation showed an encapsulation efficiency of 93.87%, particle size of 180.63 nm, and cumulative drug release of 73.94%. The developed RP-HPLC method successfully quantified Metoclopramide Hydrochloride from these nanoparticles with results well within acceptable limits, confirming its applicability for routine analysis, stability evaluation, and potential pharmacokinetic studies involving nanoparticle-based drug delivery systems.

Keywords: *RP-HPLC, Nanoparticles, Method Validation, Encapsulation Efficiency, Cumulative Drug Release*

1. INTRODUCTION

Ensuring the quality, safety, and efficacy of pharmaceutical products is crucial in the drug development process. Among various analytical techniques, Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) is widely preferred for routine analysis due to its accuracy, precision, and reproducibility. The development and validation of reliable analytical methods are essential for monitoring drug content, purity, and impurities throughout the manufacturing process. Metoclopramide Hydrochloride is a dopamine D2 receptor antagonist commonly used for managing gastrointestinal disorders such as nausea, vomiting, and gastroesophageal reflux disease (GERD) [1]. Owing to its frequent clinical application and therapeutic importance, a sensitive and validated method for its quantification in pharmaceutical dosage forms is necessary [2]. Nanoparticle-based drug delivery systems have gained prominence for improving bioavailability, stability, and controlled drug release. Natural polymers like Guar Gum, Sodium Alginate, and Chitosan are widely explored

for their biodegradable and mucoadhesive properties in such systems [3]. The present study was aimed at formulating Metoclopramide Hydrochloride nanoparticles using these polymers and optimizing the formulation through a Box-Behnken Design (BBD). The prepared formulations were evaluated for encapsulation efficiency, particle size, and drug release profile. Subsequently, a validated RP-HPLC method was employed for the estimation of Metoclopramide Hydrochloride from these nanoparticle formulations. The method was optimized and validated as per ICH Q2 (R1) guidelines for parameters like specificity, linearity, accuracy, precision, and system suitability [4]. Chemical Structure of Metoclopramide Hydrochloride are shown in Figure 1. This combined formulation and analytical study ensures the development of a reliable nanoparticulate system with accurate drug estimation

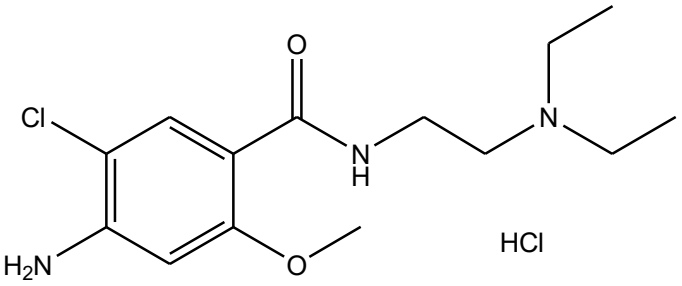


Figure 1. Chemical Structure of Metoclopramide Hydrochloride

2. MATERIALS

Metoclopramide Hydrochloride was obtained as a gift sample from a certified pharmaceutical manufacturer. Guar Gum, Sodium Alginate, and Chitosan were purchased from authorized suppliers and used without further purification. Analytical grade potassium dihydrogen phosphate (KH_2PO_4) and HPLC-grade methanol were employed throughout the study. Ultra-purified water was generated using a Milli-Q water purification system.

Preparation of Nanoparticles

Nanoparticles were prepared using a polyelectrolyte complexation method involving Guar Gum, Sodium Alginate, and Chitosan at varying concentrations as per a Box-Behnken experimental design (BBD). Accurately weighed amounts of the polymers were dissolved in distilled water. Metoclopramide Hydrochloride was dissolved separately and added dropwise to the polymeric dispersion under continuous stirring at 800 rpm. The resulting mixture was subjected to sonication for 10 minutes to reduce particle agglomeration. Nanoparticles were collected by centrifugation at 2,000 rpm for 30 minutes, washed with distilled water, and stored for further analysis [5].

Experimental Design

A three-factor, three-level Box-Behnken Design (BBD) was employed to investigate the effects of Guar Gum (A), Sodium Alginate (B), and Chitosan (C) concentrations on encapsulation efficiency, particle size, and cumulative drug release. Fifteen experimental batches were generated based on the design matrix and evaluated. The formulation composition and corresponding response values are presented in Table 1.

Table 1. Batches for Nanoparticle formulations designed by BBD

Run	Factor 1	Factor 2	Factor 3
	A: Guar Gum %	B: Sodium Alginate %	C: Chitosan %
1	2.5	1	1
2	4	1	1.5
3	2.5	3	2
4	2.5	2	1.5
5	4	2	2
6	2.5	2	1.5
7	2.5	2	1.5

8	1	3	1.5
9	4	3	1.5
10	1	1	1.5
11	1	2	1
12	2.5	3	1
13	4	2	1
14	1	2	2
15	2.5	1	2

Optimized Batch Preparation

An optimized formulation batch was prepared based on the desirability function, targeting maximum Encapsulation Efficiency, minimum Particle Size, and controlled Cumulative Drug Release. The optimal concentrations of Guar Gum, Sodium Alginate, and Chitosan were selected at a high desirability value, and the experimental results closely matched the predicted responses, confirming the model's reliability [6].

RP-HPLC Method Development and Sample Preparation

RP-HPLC Analysis

Metoclopramide Hydrochloride from the optimized nanoparticle formulation was quantified using RP-HPLC on a Waters system with an auto-sampler and UV detector. Separation was performed on a C18 column (250 mm × 4.6 mm, 5 µm) under isocratic conditions with a mobile phase of KH_2PO_4 buffer and methanol (60:40 v/v) at a flow rate of 1.0 mL/min. Detection was at 273 nm, with a 20 µL injection volume and a 10-minute run time. Data acquisition and analysis were carried out using Empower software, ensuring accurate quantification [7].

Preparation of Buffer and Mobile Phase

The buffer was prepared by dissolving 5.82g of KH_2PO_4 in 1000 mL of Milli-Q water. The solution was filtered through a 0.45 µm membrane filter and degassed by sonication. The mobile phase consisted of KH_2PO_4 buffer and methanol in a 60:40(v/v) ratio, which was similarly filtered and degassed before use [8].

Preparation of Standard Solution

A stock solution of Metoclopramide Hydrochloride (100 µg/mL) was prepared by dissolving 100 mg of the drug in 50 mL of mobile phase, followed by sonication for 10 minutes to ensure complete dissolution. The solution was then diluted to 100 mL with the same solvent. From this, a working standard solution was prepared by diluting 5 mL of the stock solution to 100mL with mobile phase [9].

Preparation of Sample from Nanoparticle Formulation

An accurately weighed quantity of the nanoparticle formulation equivalent to 150 mg of Metoclopramide Hydrochloride was dispersed in 50 mL of distilled water and transferred to a 500 mL volumetric flask. It was then mixed with 250 mL of mobile phase and sonicated for 30 minutes with intermittent shaking to ensure complete drug extraction. The volume was adjusted to 500 mL with mobile phase, filtered through a 0.45 µm membrane filter, and appropriately diluted with mobile phase prior to RP-HPLC injection [10].

3. RESULTS AND DISCUSSION

Formulation Optimization via Box-Behnken Design

A total of 15 nanoparticle formulations of Metoclopramide Hydrochloride were prepared based on a Box-Behnken Design (BBD) to evaluate the influence of three independent variables: Guar Gum (A), Sodium Alginate (B), and Chitosan (C) concentrations, on three dependent responses Encapsulation Efficiency (Y_1), Particle Size (Y_2), and Cumulative Drug Release (Y_3). The observed values for each formulation batch are provided in Table 2.

Table 2. Observed responses of Nanoparticle formulations designed by BBD.

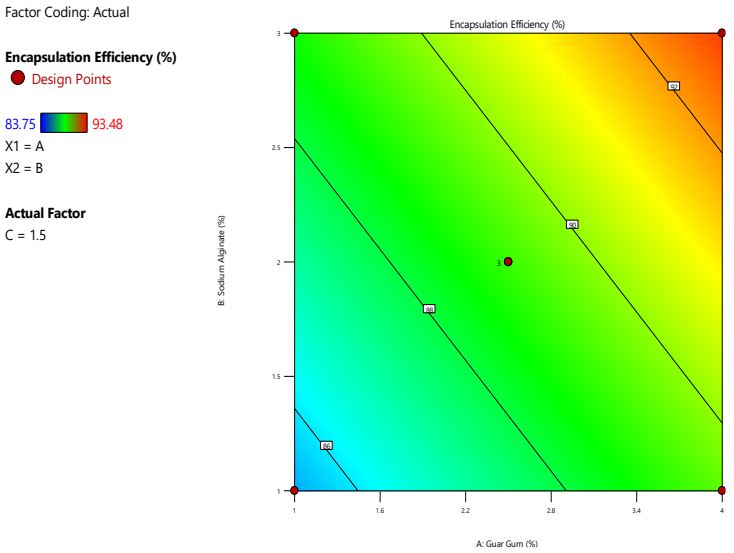
Run	Response 1	Response 2	Response 3
	EE %	Particle Size µm	CR %
1	85.02	221	65.1
2	88.47	209	69.9
3	90.05	201	72.2
4	86.53	216	67.1
5	91.08	196	73.2
6	92.47	181	75.9
7	88.85	206	70.2
8	89.95	199	71.3
9	93.48	176	78.1
10	86.89	214	66.2
11	83.75	224	64.2
12	90.48	191	74.4
13	92.02	186	75.8
14	88.03	211	71.6
15	90.02	201	73.1

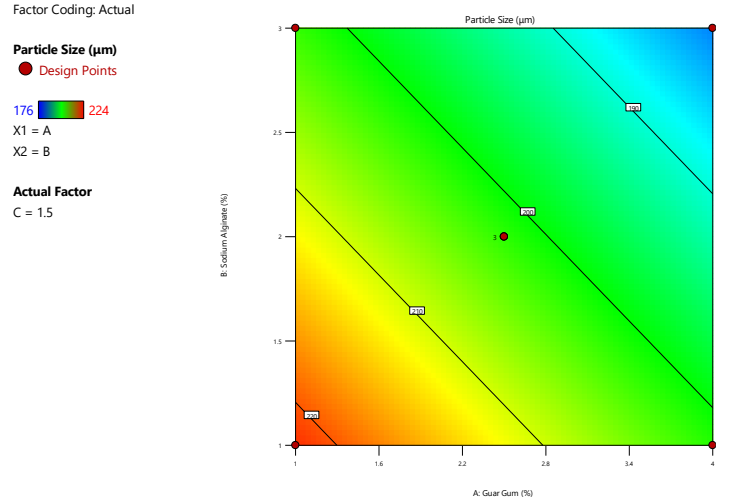
ANOVA Analysis

The formulation optimization using Box-Behnken Design and subsequent ANOVA analysis revealed that for encapsulation efficiency and particle size, the linear models were significant with p-values of 0.0129 and 0.0251, respectively, where Guar Gum (A) and Sodium Alginate (B) were significant factors ($p < 0.05$), while Chitosan (C) showed no significant effect. In the case of cumulative drug release, a 2FI model was significant ($p = 0.0123$), with Guar Gum (A) and Sodium Alginate (B) again identified as significant, while the interactions AC and BC were near significant ($p \approx 0.06–0.07$), indicating a potential synergistic influence on drug release. For all three responses, the lack of fit was non-significant ($p > 0.9$), confirming model adequacy, Results are given in Table 3 and Figure 2. These findings collectively suggest that Guar Gum and Sodium Alginate concentrations are critical formulation variables affecting encapsulation efficiency, particle size, and drug release behaviour in the prepared nanoparticulate system, supporting the suitability of the optimized formulation for effective drug delivery.

Table 3. ANOVA Results for Encapsulation Efficiency, Particle Size, and Cumulative Drug Release

Response	Model F-value	p-value (Model)	Significant Factors ($p < 0.05$)	Non-significant Factors ($p > 0.05$)	Lack of Fit F-value	p-value (Lack of Fit)
Encapsulation Efficiency	5.75	0.0129	A , B	C	0.29	0.9237
Particle Size	4.62	0.0251	A, B	C	0.21	0.9603
Cumulative Drug Release	5.95	0.0123	A, B	C, AB, AC (~0.065), BC (~0.061)	0.04	0.9991





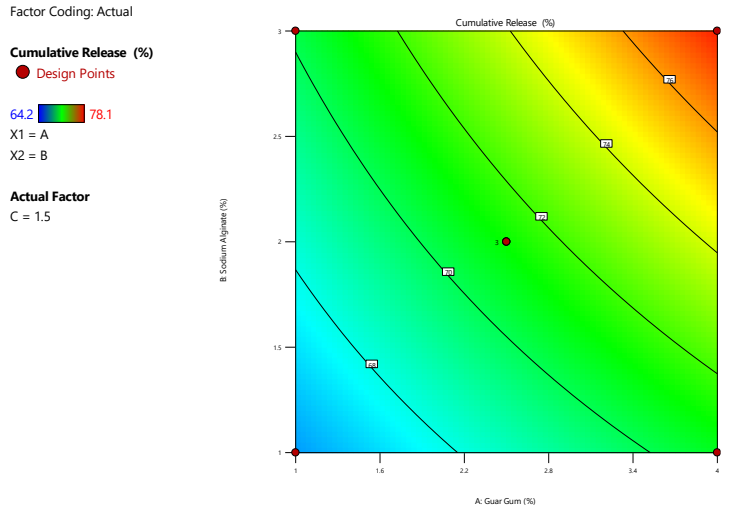


Figure 2: Contour Plot Representation of the Effect of Independent Variables on Formulation Responses

Optimized Formulation

The formulation with the highest desirability value of 0.9505 was selected as the optimized batch, achieved at Guar Gum 2.99%, Sodium Alginate 2%, and Chitosan 1.23%. The predicted encapsulation efficiency, particle size, and cumulative drug release were 93.87%, 180.63 nm, and 73.94%, respectively. Upon experimental evaluation, the observed encapsulation efficiency was 93.25%, particle size was 185.05 nm, and cumulative drug release was 71.85%. All the observed values were found to be within 5% variation of their respective predicted values, confirming the suitability and reliability of the optimized formulation as per the design model.

Specificity

Specificity of the method was determined by comparing the chromatograms of the blank, standard and sample solutions. Analysis of Metoclopramide Hydrochloride presented no interfering peaks at its retention time, thus the method is free from matrix interference. This method's selectivity was confirmed by an obtained main peak that was spectrally pure. The data of specificity is as shown in Table 4 and Figure 3, which maintains identical retention time and peak purity for both standard and sample solution.

Table 4. Specificity Data for Metoclopramide Hydrochloride

Solution Type	Retention Time (min)	Peak Purity
Blank	No interference	—
Standard Solution	6.798	1.0
Sample Solution	6.78	1.0

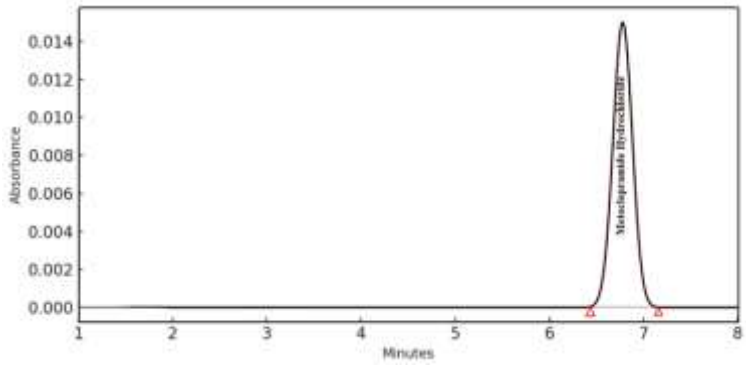


Figure 3. Chromatogram Showing Retention Peak at 6.78 Minutes

Precision

It was evaluated by analyzing six replicates of similar batch. High repeatability is indicated by the well within acceptable limits %RSD of the assay values. Second analyst performed a second (on another day and with a different column) assessment of precision at intermediate precision, which was similar. The precision and intermediate precision results respectively are summarized in Table 5 and Table 6.

Table 5. Precision of Metoclopramide Hydrochloride

Sample No.	Area	% Assay
1	987320	101.2
2	983450	101.0
3	982620	101.1
4	980330	100.9
5	981900	101.0

6	978850	100.8
Mean	—	**101.0**
%RSD	—	**0.18**

Table 6. Intermediate Precision of Metoclopramide Hydrochloride

Sample No.	Area	% Assay
1	971400	100.6
2	973100	100.7
3	970880	100.5
4	972330	100.6
5	973750	100.8
6	974100	101.0

Linearity and Range

Typically, with an excellent linearity between a wide concentration range, from 50% to 150% of the target concentration, the method was demonstrated. An analysis shows that the linearity is strong as the correlation coefficient (R^2) is greater than 0.999. The data of the Table 7 and Figure 4 are for the linearity.

Table 7. Linearity of Metoclopramide Hydrochloride

Concentration (%)	Peak Area
50%	463200
80%	740100
100%	928350
120%	1119200
150%	1396500

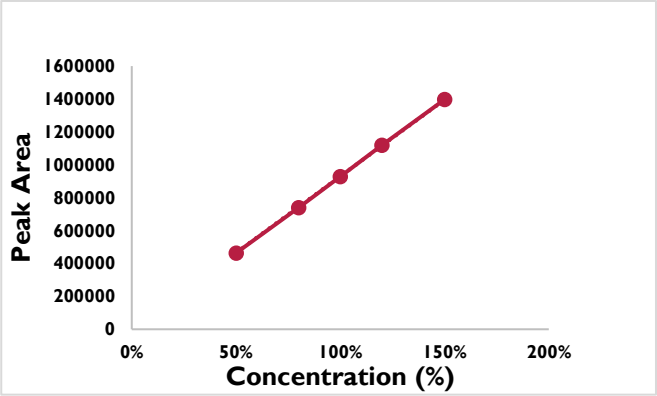


Figure 4. Linearity Curve of Metoclopramide Hydrochloride

Accuracy

Recovery studies were performed at three levels (recovery accuracy) 50%, 100%, and 150%. The accuracy of the method is confirmed by the average recovery values which ranged between 98.5% and 101.7%, within the acceptable range of 97–103%. The data in accuracy table 8 at various concentration levels is provided.

Table 8: Accuracy (Recovery) of Metoclopramide Hydrochloride

Level (%)	% Recovery
50%	98.9, 99.2, 99.4
100%	100.8, 101.2, 101.7
150%	100.5, 100.9, 101.3
Mean	**100.4**

System Suitability

The system suitability tests were carried out prior to the performance validation and the sample analyses, to ascertain that the chromatographic system was operating as required. It included retention time, tailing factor, theoretical plates and %Relative standard deviation of peak areas from the multiple injections of the standard solution. The reliability, efficiency and stability of the system under standard working conditions are proven by these tests. The peak symmetry in the results was consistent, there was high column efficiency and minimal variation in the peak areas which showed that the system was suitable for routine analysis of Metoclopramide Hydrochloride. Table 9 values are clearly acceptable criteria that are specified by the regulatory guidelines (i.e., tailing factor of less than 2.0, total theoretical plates of more than 2000, % RSD of less than 2.0), indicating that the system is well suited for the reproducible and reliable analyses.

Table 9. System Suitability Parameters for Metoclopramide Hydrochloride

Injection No.	Retention Time (min)	Peak Area	Tailing Factor	Theoretical Plates
1	6.78	987320	1.01	3100
2	6.77	983450	1.00	3125
3	6.78	982620	1.02	3095
4	6.77	980330	1.01	3080
5	6.78	981900	1.01	3110
6	6.78	978850	1.00	3078
Mean	**6.78**	—	**1.01**	**3098**
%RSD (Area)	—	**0.23**	—	—

Robustness

The developed RP-HPLC method was found robust and stable as deliberately some small variations of the chromatographic conditions were made to evaluate the method's resilience as well as reliability. To ensure that in minor change a method is still yielding acceptable results, slight change of parameters, column oven temperature, mobile phase composition, flow rate, and detection wavelength have been done. It was found that such variations have little effect on retention time, peak area, tailing factor and theoretical plates, implying the robustness of the method. However, the relative standard deviation (%RSD) values for the method were within acceptable limits even under slightly different analytical conditions, which proves that the method is stable on variations. In Table 10 and Figure 5, retention time variation due to deliberate variation of method parameters is illustrated.

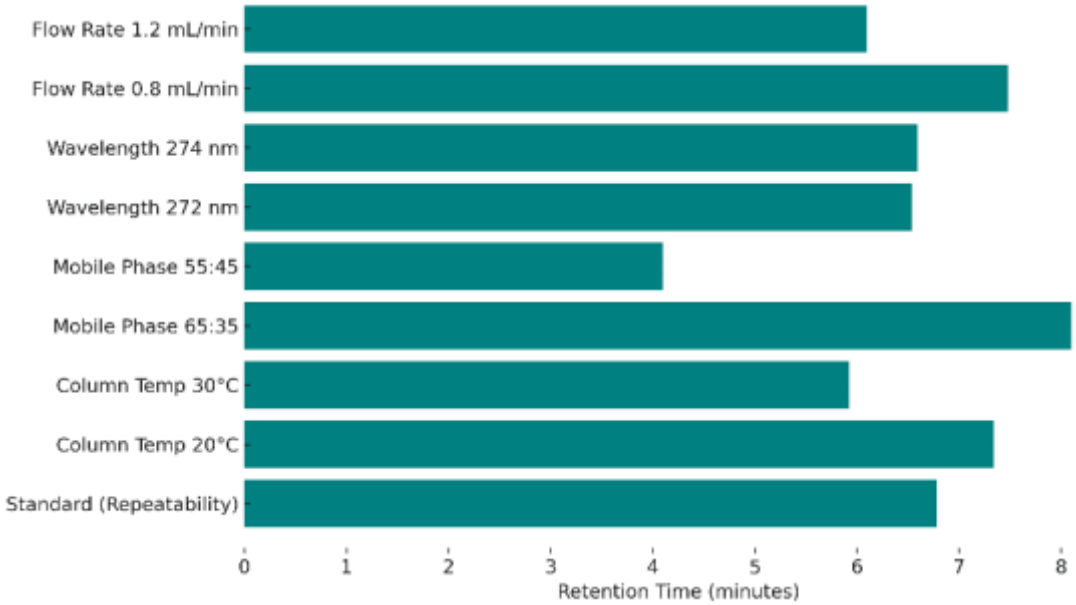


Figure 5. Retention Time under Robustness Conditions

Table 10. Robustness Parameters for Metoclopramide Hydrochloride

Condition Modified	Retention Time (min)	Tailing Factor	Theoretical Plates	%RSD	Assay (%)
Standard Condition	6.78	1.01	3105	0.23	101.0
Column Temperature: 20°C	7.34	1.28	2780	0.21	101.2
Column Temperature: 30°C	5.92	1.25	2604	0.19	100.8
Mobile Phase Ratio (65:35 buffer:methanol)	8.10	1.15	3402	0.22	99.5
Mobile Phase Ratio (55:45 buffer:methanol)	4.10	1.30	2480	0.20	99.8
Detection Wavelength – 272 nm	6.54	1.11	2756	0.18	100.7
Detection Wavelength – 274 nm	6.59	1.17	2895	0.17	101.1
Flow Rate – 0.8 mL/min	7.48	1.20	2650	0.20	100.9
Flow Rate – 1.2 mL/min	6.10	1.25	2701	0.19	100.6

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

The sensitivity of the developed RP-HPLC method was determined from the determination of the Limit of Detection (LOD) and the Limit of Quantification (LOQ). Using the signal to noise method, these parameters were calculated to be the LOD calculated at 3:1 and the LOQ at 10:1. The method showed adequate sensitivity for Metoclopramide Hydrochloride where the LOD value approached 0.36 µg / mL and LOQ about 1.56 µg / mL. The method is capable of detecting and quantifying even trace amounts of the analyte in pharmaceutical formulations and these findings indicate that the method is reliable.

Forced Degradation Studies

To test the stability indicating capability of the proposed method, Metoclopramide Hydrochloride was subjected to different stress conditions such as acid, alkaline, oxidative and thermally degraded. These studies were conducted by exposing the drug substance at: 3 % Hydrogen Peroxide (oxidative stress), Acid hydrolysis with 0.1 N Hydrochloric Acid, Alkaline hydrolysis with 0.1 N Sodium Hydroxide and Thermal degradation at 105°C. The degradation peaks were clearly separated

from the main peak of Metoclopramide Hydrochloride under all stress conditions and did not interfere with Metoclopramide Hydrochloride retention time or peak purity thus proving the method stability indicating nature. The results (Table 11) of degradation indicate that the Metoclopramide Hydrochloride exhibits slight to moderate degradation upon stress, and the method effectively resolves the drug peak from any degradation product.

Table 11. Result of Forced Degradation Study for Metoclopramide Hydrochloride

Stress Condition	Average Peak Area	% Assay Remaining
Acidic Hydrolysis	870250	91.10%
Alkaline Hydrolysis	874320	92.50%
Oxidative Degradation	765140	80.30%
Thermal Degradation	872580	92.00%

4. CONCLUSION

The present study successfully developed and optimized Metoclopramide Hydrochloride-loaded nanoparticles using a Box-Behnken Design approach. The optimized formulation, achieved at Guar Gum 2.99%, Sodium Alginate 2%, and Chitosan 1.23%, demonstrated desirable characteristics with an encapsulation efficiency of 93.87%, particle size of 180.63 nm, and cumulative drug release of 73.94%. The RP-HPLC method developed for the quantitative estimation of Metoclopramide Hydrochloride from the nanoparticle formulation proved to be simple, accurate, precise, and robust, with a retention time of 6.78 minutes. The method exhibited satisfactory specificity, linearity, sensitivity, and stability, making it suitable for both routine quality control and stability-indicating analysis. Overall, the combined formulation and analytical approach offers a reliable strategy for nanoparticulate drug delivery system evaluation and pharmaceutical analysis of Metoclopramide Hydrochloride.

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Conflict of Interest

The authors declare that there is no conflict of interest associated with this research work.

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