

Article

Determination of Sodium Carboxymethyl Cellulose in Diluent of Risperidone Injectable Suspension via Dische Reaction

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Abstract

Sodium carboxymethyl cellulose (NaCMC) is a commonly used pharmaceutical excipient; however, no analytical method has been specifically reported for its determination in the diluent of risperidone injectable suspension. In this study, a simple and cost-effective spectrophotometric method based on the Dische reaction was developed and validated for the quantification of NaCMC. Critical reaction parameters were investigated and optimized using a design of experiments (DoE) approach. The method was validated according to ICH guidelines and demonstrated satisfactory specificity, linearity, accuracy, and precision over the concentration range of 25–150 µg/mL. Recovery values ranged between 98.5 and 101.3%, while repeatability and intermediate precision (expressed as %RSD) was less than 0.7%. The robustness of the derivatization procedure was examined using Monte Carlo simulations and capability analysis. The environmental performance of the proposed method was assessed using the environmental performance and practicality index and analytical green star area tools. The method was successfully applied to the analysis of commercial batches of risperidone injectable suspension diluent. The proposed assay offers a reliable, economical, and straightforward alternative to more complex analytical techniques and is suitable for routine quality control determination of NaCMC in pharmaceutical formulations.

Keywords: sodium carboxymethyl cellulose; Dische reaction; determination; risperidone; spectrophotometry



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1. Introduction

Risperidone injectable suspension is a long-acting antipsychotic formulation widely used in the management of schizophrenia and related psychiatric disorders. As a depot formulation, it is designed to provide sustained therapeutic drug concentrations over an extended period, thereby reducing the frequency of administration and improving patient adherence compared with daily oral medications [1,2]. The effectiveness and safety of risperidone injectable suspension depend not only on the active pharmaceutical ingredient (API) but also on the careful design of the formulation components that ensure proper reconstitution, administration, and controlled drug release following injection [3]. Among these excipients, NaCMC is commonly incorporated into the diluent as a viscosity-enhancing agent, contributing to the uniform dispersion of microparticles prior to administration.

Accurate quantification of sodium carboxymethyl cellulose in pharmaceutical formulations is essential for quality control, batch-to-batch consistency, and regulatory compliance.

However, the determination of NaCMC can be challenging because it is a polymeric carbohydrate derivative that lacks chromophoric properties. Various approaches have been developed for the determination or characterization of this compound including nuclear magnetic resonance [4], two-dimensional high pressure liquid chromatography (HPLC) [5], UV spectrophotometry (via reaction with phenol in presence of H_2SO_4) [6], fluorescence [7], and Rayleigh scattering spectrometry [8]. Among these approaches, colorimetric methods have attracted interest as practical, cost-effective, and relatively simple alternatives for routine analysis.

The Dische reaction is a well-established colorimetric assay that involves the reaction of carbohydrate derivatives with sulfuric acid and specific reagents to produce a colored complex measurable by UV–Vis spectrophotometry [9]. This reaction has been widely employed for the detection and quantification of polysaccharides and carbohydrate-containing compounds due to its sensitivity, simplicity, and reproducibility. By exploiting the carbohydrate backbone of NaCMC, the Dische reaction offers a promising approach for its quantitative determination in complex pharmaceutical matrices. Compared with more sophisticated instrumental techniques, this approach requires minimal sample preparation, utilizes readily available laboratory equipment, and is well suited for routine quality control environments.

To the best of our knowledge, no analytical method has been specifically reported for the determination of NaCMC in risperidone injectable suspension diluent. The present study aims to develop and evaluate a Dische reaction-based analytical method for the determination of NaCMC in the diluent of risperidone injectable suspension. The effect of the reaction parameters on the UV absorbance of the derivative was investigated using DoE. Method performance was assessed in terms of specificity, linearity, accuracy, precision, and suitability for routine quality control applications. The successful implementation of this method would provide a reliable, economical, and efficient analytical tool for routine quality control of NaCMC in pharmaceutical formulations and could serve as a practical alternative to more complex and resource-intensive analytical techniques.

2. Materials and Methods

2.1. Reagents and Solutions

NaCMC (>99.5%) was purchased from Ashland (Wilmington, NC, USA) while diphenylamine was supplied from ThermoScientific (Waltham, MA, USA). Concentrated HCl and glacial acetic acid were obtained from Honeywell (Charlotte, NC, USA) and Fisher (Pittsburgh, PA, USA). Distilled water was obtained from STILMAS S.p.A. (Settala, Italy), a purified water production plant. All other chemicals were analytical grade or above.

A stock NaCMC standard solution ($500\text{ }\mu\text{g mL}^{-1}$) was prepared by dissolving an accurately weighed amount of reference standard in purified water preheated to $40\text{ }^\circ\text{C}$. The solution was stirred continuously until complete dissolution of NaCMC was achieved, allowed to cool to room temperature, and then diluted to volume with purified water.

The diphenylamine reagent solution (90 mM) was prepared by dissolving the appropriate amount of diphenylamine in 150 mL of glacial acetic acid under continuous stirring. After complete dissolution, 90 mL of concentrated hydrochloric acid was added slowly while maintaining stirring.

2.2. Instrumentation

All UV–visible spectrophotometric analyses were conducted using a PerkinElmer Spectrum 100 UV–Vis spectrophotometer (PerkinElmer, Waltham, MA, USA). The absorbance of the reaction solutions was recorded at 635 nm, corresponding to the wavelength of maximum absorption (λ_{max}) of the chromophore generated through the Dische reaction.

2.3. Analytical Procedure

A 1.0 g portion of risperidone injectable suspension diluent was accurately weighed and transferred to a 200 mL volumetric flask, diluted to volume with purified water, and mixed thoroughly. An aliquot of 2.0 mL of the resulting solution (or purified water for the blank) was transferred to a glass reaction vial with PTFE caps, followed by the addition of 5.0 mL of diphenylamine reagent solution. After gentle mixing to ensure homogeneity, the vial was heated in a preheated oven at 100 °C for 2 h to promote complete formation of the colored reaction product. All sample preparation steps were performed in a fume hood while wearing appropriate personal protective equipment. The reaction mixture was subsequently cooled to room temperature, and its absorbance was measured at 635 nm against a reagent blank. Calibration standards, test samples, and blank solutions were prepared and analyzed using the same procedure.

2.4. System Suitability & Check Standard

A working standard solution containing 0.1 mg/mL NaCMC was prepared by transferring 4.0 mL of the stock standard solution (500 µg/mL) into a 20 mL volumetric flask and diluting to volume with purified water. An independently prepared solution of the same concentration was used as a check standard. The working standard served for system suitability evaluation, whereas the check standard was employed to verify the consistency and reliability of method performance throughout the analytical sequence.

2.5. Central Composite Design (CCD)

A response surface methodology based on a face-centered central composite design (FC-CCD, $\alpha = 1$) was employed to optimize the experimental conditions of the proposed method. Preliminary studies identified diphenylamine concentration (A), reaction time (B), and reaction temperature (C) as the critical factors influencing the absorbance of the NaCMC-diphenylamine reaction product. The investigated ranges were 80–110 mM for diphenylamine concentration, 1.5–2.5 h for reaction time, and 90–110 °C for reaction temperature. These variables were evaluated at three levels to investigate their linear, quadratic, and interaction effects on the analytical response.

The experimental design was generated using Design-Expert[®] software (Version 13, Stat-Ease Inc., Minneapolis, MN, USA) and consisted of 20 runs, including 8 factorial points, 6 axial points, and 6 center points. Replicate center-point experiments were incorporated to estimate pure error and assess the adequacy and reproducibility of the fitted model. Absorbance at 635 nm was selected as the response variable and used to evaluate the influence of the investigated factors on method performance. The experimental design matrix and the corresponding absorbance responses are presented in Table 1.

2.6. Method Validation

The analytical performance of the proposed method was evaluated through a validation study according to ICH guideline that included assessment of specificity, linearity, repeatability, intermediate precision, accuracy, and reference solution stability [10]. These performance characteristics were investigated to demonstrate the suitability, reliability, and consistency of the method.

2.6.1. System Suitability Test

System suitability criteria were established during method development and subsequently verified during method validation to ensure adequate system performance prior to sample analysis. The acceptance criteria included: (i) a blank absorbance response not exceeding 5% of the system suitability standard response; (ii) a relative standard deviation

(%RSD) of $\leq 5.0\%$ for replicate measurements of the system suitability standard solution; (iii) a %RSD of $\leq 5.0\%$ for the combined absorbance measurements of the system suitability standard and quality control (QC) check standard solutions; (iv) a percent difference of $\leq 5.0\%$ between the absorbance values obtained for the system suitability standard and the independently prepared QC check standard solution. These criteria were used to verify instrument performance, method repeatability, and consistency of standard preparation throughout the analytical sequence.

Table 1. FC-CCD for the optimization of derivatization conditions.

Run No	C(Diphenylamine) (mM) (Factor A)	Reaction Time (h) (Factor B)	Reaction Temperature (°C) (Factor C)	Absorbance (635 nm)
1	90	2	95	0.3778
2	90	1.5	95	0.1841
3	90	2	95	0.3824
4	100	2.5	80	0.0472
5	90	2	80	0.0283
6	80	1.5	110	1.0103
7	100	1.5	80	0.0171
8	100	1.5	110	1.0674
9	100	2	95	0.3277
10	90	2	95	0.3426
11	80	2.5	110	1.4062
12	90	2	95	0.3404
13	80	1.5	80	0.019
14	90	2	95	0.3359
15	90	2	95	0.342
16	90	2	110	1.3921
17	100	2.5	110	1.5134
18	90	2.5	95	0.4005
19	80	2	95	0.3825
20	80	2.5	80	0.0498

2.6.2. Specificity

The specificity of the method was demonstrated by comparing the UV spectra of solvent, placebo and sample solutions.

2.6.3. Linear Range

A linear relationship between analyte concentration and UV absorbance was evaluated over the target concentration range of 25–150 µg/mL. Calibration standards were prepared by appropriate dilution of the NaCMC stock standard solution to six concentration levels spanning the intended analytical range. Linearity was assessed by least-squares linear regression analysis. The acceptance criteria for linearity were a correlation coefficient (r^2) of at least 0.99 and a 95% confidence interval for the regression intercept that included zero, thereby confirming the absence of significant systematic bias at the origin. These criteria were used to demonstrate the proportionality of the analytical response across the specified concentration range.

2.6.4. Quantitation Limit

The sensitivity of the method was evaluated by estimating the limit of detection (LOD), limit of quantification (LOQ) and lower limit of quantification (LLOQ). The LOD was determined from the calibration model using the relationship $Y_{LOD} = \text{intercept} + 3 \times \text{residual standard deviation}$, where the residual SD was obtained from the ANOVA of the

regression model [11]. The corresponding concentration value (X_{LOD}) was calculated by back-calculation into the calibration equation. The LOQ was estimated as $3.3 \times$ the LOD concentration, while the LLOQ was defined as the lowest concentration level included in the validated calibration range.

2.6.5. Repeatability

Precision was established by analyzing six risperidone diluent solutions ($n = 6$) where the % RSD of the NaCMC content should be less than 5%.

2.6.6. Reference Stability

The stability of the reference standard and sample solutions was determined by storing them at room temperature up to 48 h. It should be noted that each stability timepoint, blank, standards and samples solutions are the same solutions prepared and measured at zero time point. The % difference in NaCMC content of stability sample solution of each time interval should be less than 5.0 compared to sample solution at zero time.

2.6.7. Intermediate Precision

The results obtained from the two different precision analyses in repeatability conditions were further evaluated to establish the precision of the method in intermediate precision conditions. Two independent analyses were performed by two different analysts on consecutive days using different batches of reagents, as well as independently prepared stock and working standard solutions. Throughout these experiments, the same sample batch and the same UV-Vis spectrophotometer were used. The %RSD of the NaCMC content determined over 12 sample solutions (combined inter and intra runs) should be less than 10%.

2.6.8. Accuracy

Accuracy is the closeness of a measured value to the true or accepted value of the quantity being measured. The accuracy test was performed using analysis results obtained from the analyte addition-recovery application on placebo risperidone diluent sample at three concentration levels of 50, 100 and 125% of the specification limit. The %recovery should be ranged between 95 and 105% while the %RSD of the recovery at each accuracy level must be less than 5%.

3. Results and Discussion

3.1. Method Principle

Under strongly acidic conditions, NaCMC undergoes acid-catalyzed hydrolysis of its β -(1 $\rightarrow$ 4)-glycosidic bonds to produce glucose and partially carboxymethylated glucose derivatives. These monosaccharides are subsequently dehydrated to form 5-hydroxymethylfurfural, which serves as the principal chromogenic intermediate. The activated aldehyde group of 5-HMF condenses with diphenylamine through acid-catalyzed electrophilic substitution and subsequent oxidation/condensation reactions to produce a highly conjugated blue chromophore exhibiting maximum absorbance near 600–635 nm [9,12].

3.2. Optimization of the Derivatization Conditions

One of the primary objectives of this study was to investigate and optimize the derivatization conditions affecting the formation of the NaCMC–diphenylamine reaction product. According to the literature, the Dische reaction requires heating under acidic conditions to achieve efficient color development [9]. The concentration of diphenylamine (80–100 mM) and the reaction time (1.5–2.5 h) were established based on preliminary

experiments. To achieve this, a FC-CCD was built, enabling the evaluation of linear, interaction, and quadratic effects of the selected factors [13,14].

The ANOVA results (Table 2) revealed that reaction time (B) and reaction temperature (C) had the most significant influence on the absorbance response, indicating their critical role in the derivatization process. Both factors exhibited positive effects on the formation of the colored derivative, whereas diphenylamine concentration (A) was not statistically significant within the investigated range ($p > 0.05$). A significant interaction between reaction time and temperature ($B \times C$) was observed, demonstrating that the effect of one factor depended on the level of the other. In addition, the quadratic terms B^2 and C^2 significantly contributed to the model, confirming the presence of curvature in the response surface.

Table 2. ANOVA table for the absorbance of NaCMC-diphenylamine derivative.

Source	Sum of Squares	Df ¹	Mean Square	F-Value	p-Value	
Model	4.59	5	0.9171	646.67	<0.0001	significant
B-Reaction time	0.1253	1	0.1253	88.33	<0.0001	
C-Reaction temp	3.88	1	3.88	2735.15	<0.0001	
BC	0.0762	1	0.0762	53.76	<0.0001	
B^2	0.0136	1	0.0136	9.61	0.0078	
C^2	0.3979	1	0.3979	280.61	<0.0001	
Residual	0.0199	14	0.0014			
Lack of Fit	0.0177	9	0.0020	4.56	0.0548	not significant
Pure Error	0.0022	5	0.0004			
Cor Total	4.61	19				

¹ degree of freedom.

The fitted quadratic model exhibited excellent predictive capability, with r^2 and adjusted r^2 values of 0.9957 and 0.9941, respectively. Furthermore, the lack-of-fit test was not significant ($p > 0.05$), indicating that the model adequately described the experimental data [15]. The effects of the investigated variables on absorbance are illustrated by the three-dimensional response surface plots shown in Figure 1.

A normal distribution of the residuals (Figure 2), which indicates good agreement between the experimental and predicted results. Cook's distance is used to identify observations that have a disproportionately large influence on the fitted regression model [16]. As shown in Figure 2C, all observations exhibited Cook's distance values below the critical limit of 0.9357, indicating the absence of influential outliers. Although runs 5, 6, 11, 16, and 18 showed relatively higher influence compared with the remaining experiments, their values remained within acceptable limits. These results demonstrate that no single experimental run exerted an undue effect on model fitting, confirming the robustness and reliability of the developed regression model.

The mathematical model related to derivatization is elucidated through the generated second-order polynomial equation. The equation explains how, and to what extent, the independent variables and their binary interactions affect the derivative absorbance. In the equation, the magnitudes of the coefficients, along with their minus and plus signs, define both the positive and negative impacts and contributions of the experimental parameters to the absorbance. The mathematical expression is given below:

$$\text{Abs} = 11.536 + 0.0313B - 0.282C + 0.013BC - 0.261B^2 + 0.001C^2 \quad (1)$$

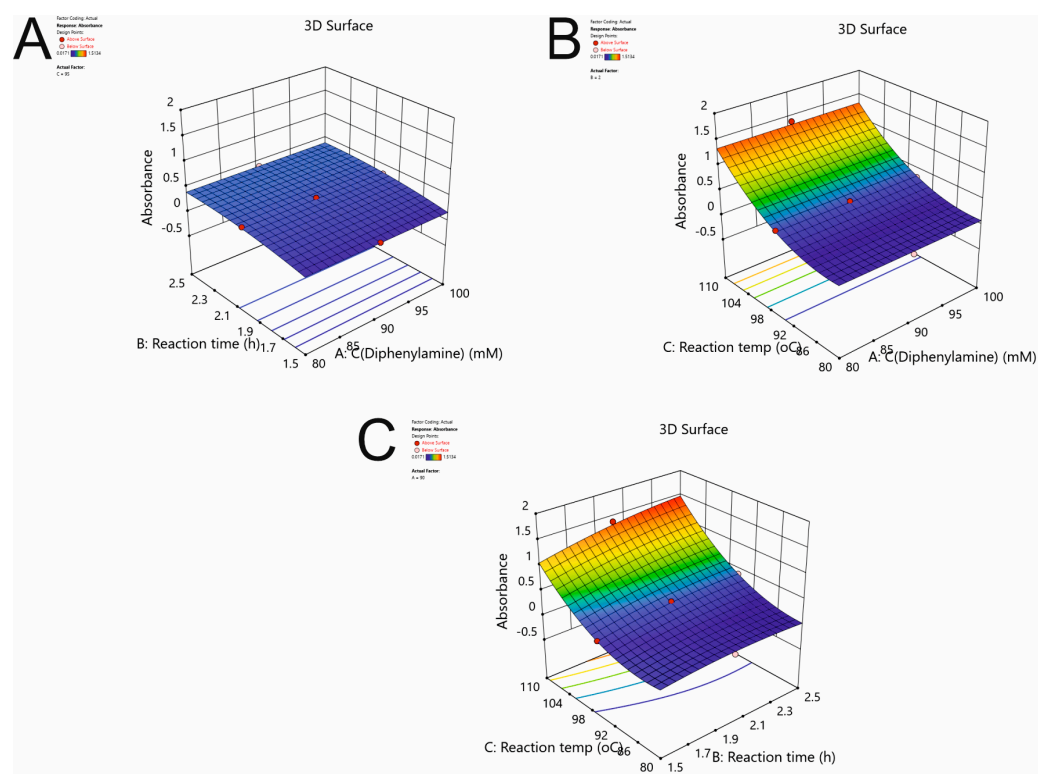


Figure 1. 3D plots of the effect of (A) reaction time and diphenylamine concentration, (B) reaction temperature and diphenylamine concentration, and (C) reaction time and reaction temperature on the derivative absorbance.

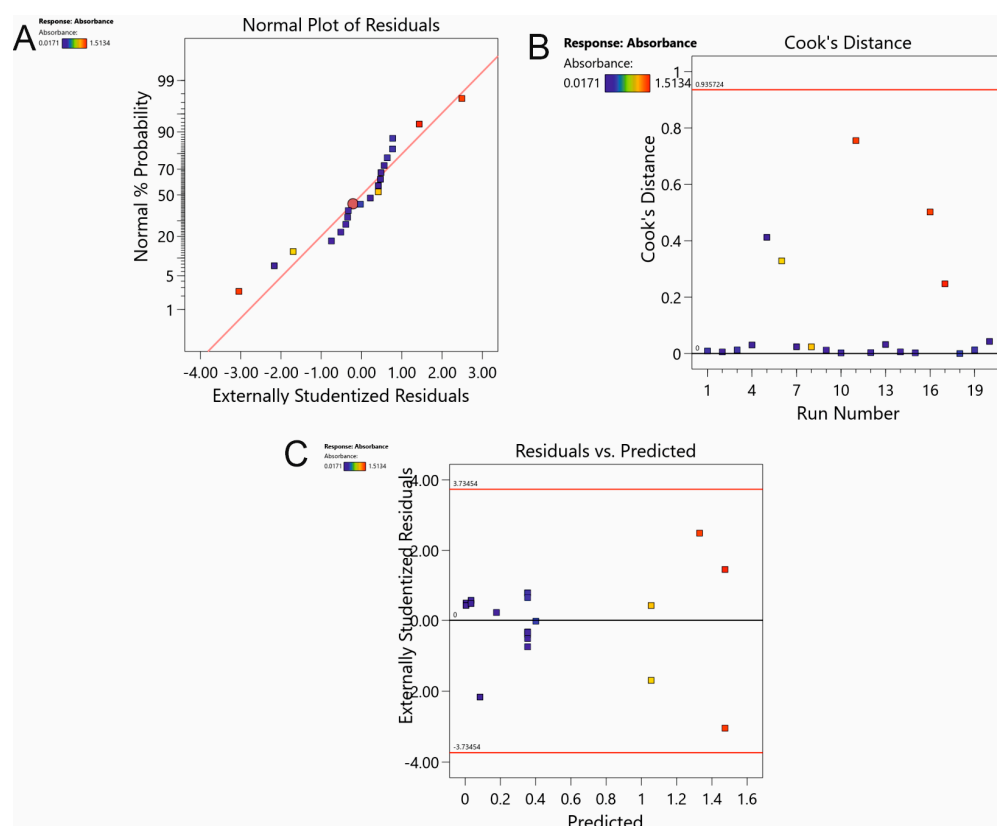


Figure 2. (A) Normal probability, (B) Cook's distance, and (C) the residuals vs. predicted plots for the derivative absorbance. Where squares and red lines represent the experimental runs and reference line, respectively.

The optimal derivatization conditions were established using Derringer's desirability function with the objective of maximizing the absorbance response of the derivative [17]. To ensure safe operation and avoid potential risks associated with heating highly acidic solutions, the reaction temperature was constrained to 100 °C during the optimization process. Under these conditions, a composite desirability value of 0.417 was obtained, indicating an acceptable compromise between analytical performance and operational practicality.

The optimized conditions consisted of a diphenylamine concentration of 90 mM, a reaction time of 2 h (rounded value), and a reaction temperature of 100 °C (Figure 3). The resulting desirability reflects the balance between maximizing absorbance and minimizing derivatization time, two objectives that exhibit opposing effects on method performance. The predictive capability of the optimization model was subsequently verified through six replicate experiments performed at 100% of the target concentration. The observed responses were in good agreement with the model predictions, with predicted-to-observed absorbance ratios ranging from 90.1% to 104.0% within the 95% CI, thereby confirming the adequacy and reliability of the proposed model.

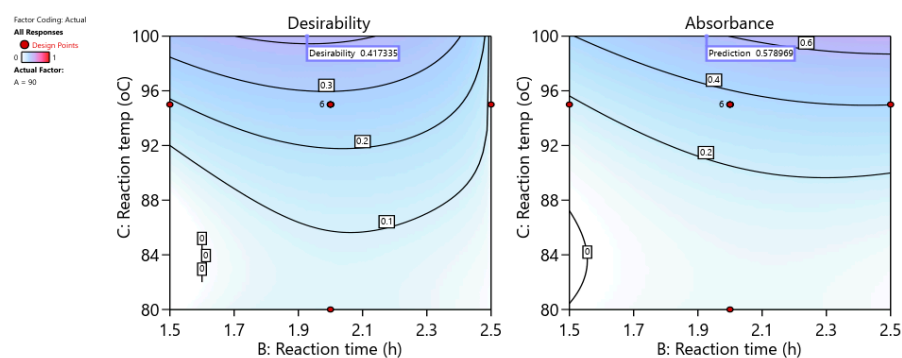


Figure 3. Desirability (left) and absorbance (right) plots of the reaction temperature and reaction time.

3.3. Method Validation

When developing an analytical method, one of the most desired and primarily targeted features of the method is selectivity [18]. The selectivity of the proposed spectrophotometric method was evaluated by comparing the UV–Vis absorption spectra obtained from the blank solution, placebo solution, NaCMC reference standard solution, and risperidone injectable suspension diluent sample after derivatization with diphenylamine.

As shown in Figure 4, the NaCMC standard and sample solutions exhibited nearly identical spectral profiles, with characteristic absorption maxima at approximately 635 nm, confirming the formation of the same chromogenic derivative in both solutions. In contrast, the blank and placebo solutions showed negligible absorbance throughout the investigated wavelength range and did not exhibit any absorption bands at the characteristic wavelengths of the NaCMC derivative. These results demonstrate the absence of interference from excipients, reagents, or other matrix components and confirm that the method is sufficiently selective for the determination of NaCMC in the diluent of risperidone injectable suspension.

The linearity of the proposed method was evaluated using six calibration levels spanning a concentration range of 24–146 µg/mL NaCMC (Figure 5). A strong linear relationship between absorbance and NaCMC concentration was observed across the investigated range. Linear regression analysis yielded a correlation coefficient (r^2) of 0.9956, demonstrating excellent proportionality of the analytical response. The validity of the regression model was further confirmed by residual analysis, which showed no systematic trends. The residuals were found to be normally distributed, as indicated by the Shapiro–Wilk test ($p > 0.05$), supporting the suitability of the linear model. All calibration levels were analyzed in triplicate to ensure the reliability and reproducibility of the generated data.

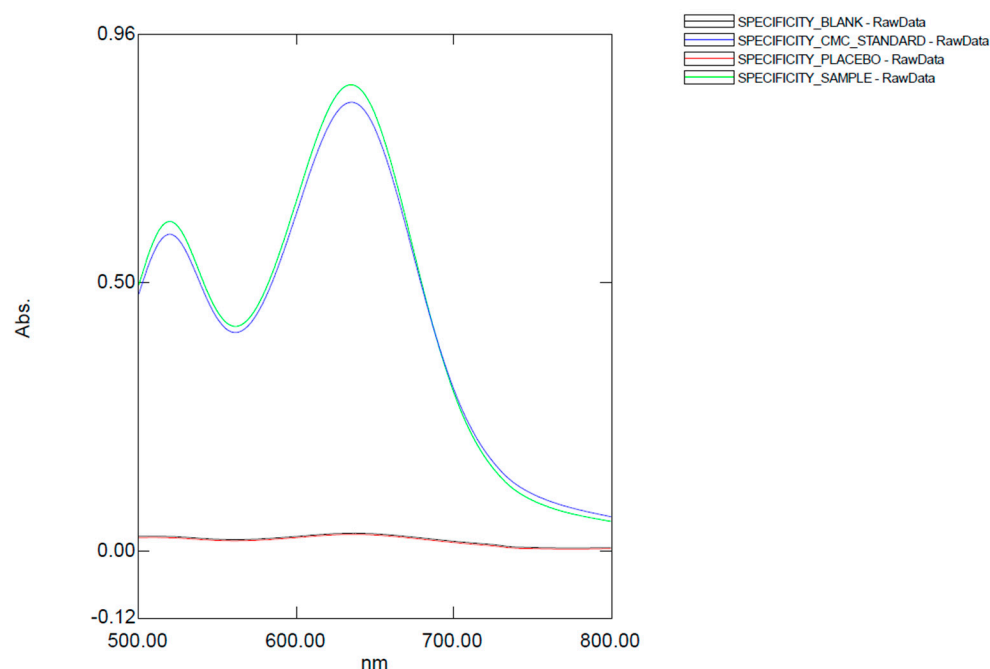


Figure 4. UV–Vis spectra of blank solution (black line), NaCMC standard solution (100 µg/mL) (blue line), placebo sample (red line) and risperidone diluent test sample (green line).

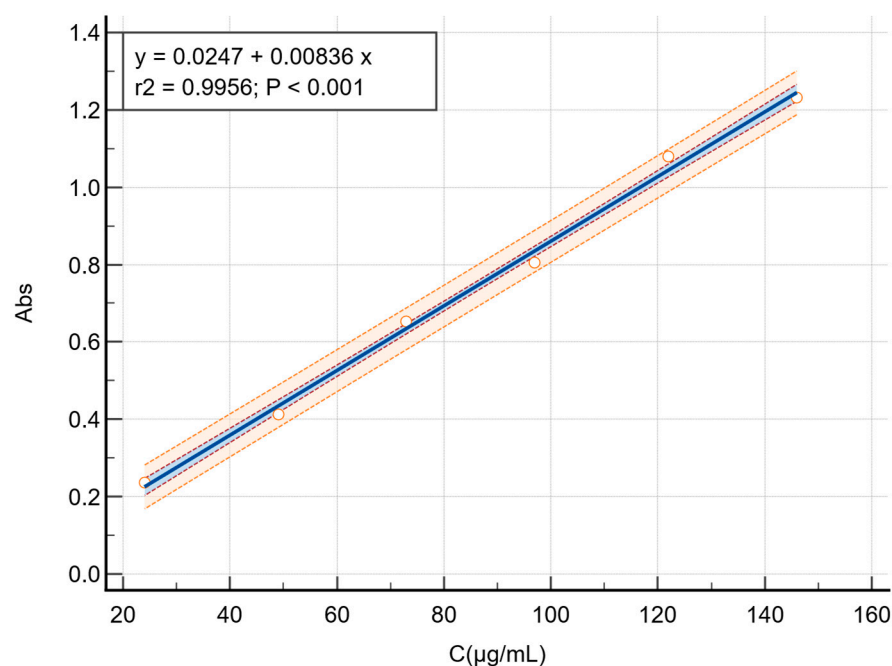


Figure 5. Linear regression analysis of the NaCMC calibration data obtained by the proposed method. The regression line is shown together with the 95% confidence interval for the mean response and the 95% prediction interval for individual observations.

The intra-day and intermediate precision were evaluated at 100% specification limit of the analyte. Intermediate precision was assessed by a second analyst on a different day. Adequate values for intra-day and intermediate precision were obtained at less than 1.3% in all cases (Table 3), which are in compliance with the acceptance criteria of 5% (for intraday precision) and 10% (for intermediate precision), respectively. These results demonstrate that the method is reproducible under varied analysts, confirming its suitability for routine analysis.

Table 3. Intra-day and intermediate precision of the proposed method.

Risperidone Diluent Sample	CMC Content (mg/mL)	Average CMC Content (mg/mL)	% RSD (n = 6)
Intra-day precision			
Sample A	22.4	22.3	1.2
Sample B	22.4		
Sample C	22.5		
Sample D	22.0		
Sample E	21.9		
Sample F	22.5		
Intermediate precision			
Sample A	21.8	21.9	0.6
Sample B	21.8		
Sample C	22.0		
Sample D	22.0		
Sample E	21.8		
Sample F	21.9		
Cumulative intra- and inter-precision	-	21.9	1.3

The LOD of the analyte was estimated as $Y_{LOD} = \text{intercept} + 3 \times \text{residual SD}$ and was found to be 6 µg/mL. The LOQ and LLOQ were 20 and 24 µg/mL, respectively.

The accuracy of the method was evaluated by the analysis of three sample solutions containing NaCMC at three concentration levels corresponding to 50%, 100% and 125%. According to the results of Table 4, the method demonstrated excellent accuracy across the evaluated concentration range. Mean recoveries at the 50%, 100%, and 125% spiking levels were 98.5%, 101.3%, and 100.9%, respectively, all of which complied with the predefined acceptance criterion of 95.0–105.0%. The precision of the recovery measurements was also satisfactory, with %RSD values being less than 0.7%. These results confirm that the proposed method provides accurate quantification of NaCMC in the sample matrix and delivers consistent performance over the studied analytical range.

Table 4. Accuracy results of the proposed method.

Accuracy Sample Solution	%R	Average %R	%RSD (<i>n</i> = 3)
50% level-1	99.0	98.5	0.4
50% level-2	98.4		
50% level-3	98.2		
100% level-1	100.8	101.3	0.7
100% level-2	102.1		
100% level-3	101.0		
125% level-1	100.8	100.9	0.4
125% level-2	100.5		
125% level-3	101.3		

Finally, the stability of standard and test sample solutions was investigated up to 48 h at room temperature (20–25 °C) and analyzed at predetermined intervals of 4, 24, and

48 h. The stability was assessed by calculating the absolute difference in NaCMC content (mg/mL) at each time point relative to the initial value measured at time zero. The % difference for the content in test solutions at a specified time interval compared to initial content is calculated by the following formula:

$$\% \text{ Difference} = \left| \frac{\text{Content}(t) - \text{Content}(t_0)}{\text{Content}(t_0)} \right| \times 100 \quad (2)$$

where content (t_0) and (t) is the average content of NaCMC in sample solution at zero time and the specified time interval.

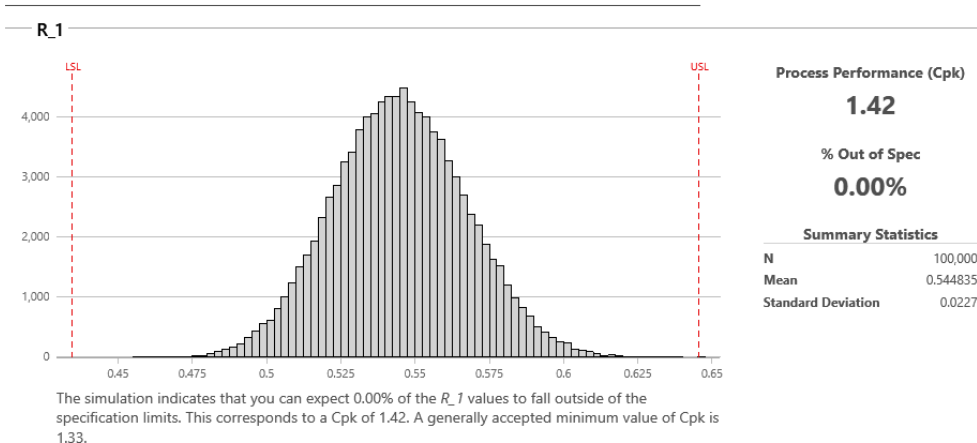
The differences observed ranged from 0.4% to 1.5% over the 48 h study period (Table S1). These minimal changes demonstrate that the analyte remained stable under the storage conditions investigated.

The robustness of the derivatization procedure was evaluated using Monte Carlo simulations combined with process capability analysis [19]. Conventional robustness studies evaluate the effect of deliberately varying one or a limited number of parameters at a few predefined levels. In contrast, Monte Carlo simulation provides a probabilistic assessment of method performance by simultaneously considering the combined variability of multiple critical method parameters over numerous simulated experiments. This approach provides a more comprehensive evaluation of method robustness and reliability while making full use of the predictive DoE model.

A total of 100,000 simulation iterations were performed to assess the impact of normal variations in critical method parameters on the analytical response. Simulated absorbance data were subsequently used to estimate the process capability index (Cpk). Acceptance limits for the absorbance response were defined as $\pm 10\%$ of the predicted value obtained from the optimization study (Section 3.1).

The simulations were conducted using the optimized conditions of 2 h reaction time and 100 °C reaction temperature as the target values, with standard deviations of 0.01 h and 0.4 °C, respectively, to reflect realistic operational variability. The resulting capability analysis histograms for NaCMC are presented in Figure 6. The calculated Cpk value was 1.42, exceeding the commonly accepted criterion of 1.33 for capable analytical processes.

Simulation Results



Next Steps

Although the Cpk is above the generally accepted value, you may want to perform a **Sensitivity Analysis**, which demonstrates how changes to the variation of the inputs affect the variation of R_1 .

[Sensitivity Analysis](#)

Figure 6. Probabilistic distribution of the absorbance values of the NaCMC derivative during Monte Carlo simulation experiments.

3.4. Method Application

The developed method was successfully applied to the analysis of several batches of risperidone injectable suspension diluent (Table 5). Across fifteen batches analyzed the determined NaCMC content was ranged between 21.5 and 23.1 mg/mL with an average of 22.2 ± 0.4 mg/mL (%RSD = 2.0%). The assay results demonstrated the suitability of the method for routine quality control testing.

Table 5. Sample analysis.

Sample	NaCMC Content (mg/mL)
Sample 1	22.5
Sample 2	22.5
Sample 3	22.0
Sample 4	22.5
Sample 5	22.1
Sample 6	22.1
Sample 7	21.8
Sample 8	21.5
Sample 9	21.7
Sample 10	22.2
Sample 11	21.8
Sample 12	21.9
Sample 13	22.8
Sample 14	23.1
Sample 15	22.3

3.5. Comparison with Other Published Methods

The analytical performance of the proposed method was compared with previously reported ones for NaCMC determination in different sample matrices (Table 6). The proposed method provides satisfactory analytical figure of merits in terms of linear range, LOD and accuracy. Although previously reported methods achieved lower detection limits (0.028 mg/L [8] or 58 µg/L [7], depending on the analytical approach), these methods were developed for different sample matrices, such as dairy products and tobacco, where lower analyte concentrations are generally expected. In contrast, the present method was specifically designed for the quality control of risperidone injectable suspension diluent, in which NaCMC is present at considerably higher concentrations. Therefore, the achieved sensitivity is sufficient for its intended application.

Regarding accuracy, the proposed method demonstrated excellent recoveries that are comparable to or better than those reported in the literature. Moreover, the method employs a simple colorimetric reaction followed by UV–Vis detection, avoiding the need for more specialized instrumentation such as fluorescence or resonance Rayleigh scattering spectrometry. This simplicity, combined with satisfactory analytical performance, makes the proposed procedure suitable for routine quality control laboratories, where rapid, reliable, and cost-effective analysis is required.

3.6. Greenness Assessment Using the Unified MA Tool

The environmental sustainability and practical applicability of the proposed method were evaluated using the Environmental Performance and Practicality Index (EPPI) [20] and

the Analytical Green Star Area (AGSA) [21] tools. EPPI provides an integrated assessment of both environmental impact and operational practicality. The environmental component considers factors related to sample preparation, instrumentation, reagent consumption, and waste generation, while the practicality component evaluates the overall suitability of the method for routine laboratory implementation.

Table 6. Comparison of the proposed method with other published approaches for the determination of NaCMC.

Sample	Method Principle	Detection	Linear Range	LOD	RR ¹ (%)	Ref.
Yogurt, ayran	Complexation using CuSO ₄ and reaction phenol in the presence of H ₂ SO ₄	UV–Vis spectrophotometry (@490 nm)	0.1–2% (1000–20,000 µg/mL)	0.03% (300 µg/mL)	98–100%	[6]
Tobacco	Indirect determination after its reaction with acridine yellow or acridine orange	Fluorescence ($\lambda_{ex}/\lambda_{em}$ = 440/505 nm)	20–4000 µg/L (using acridine yellow) 20–7000 µg/L (using acridine orange)	58 µg/L (using acridine yellow) 157.2 µg/L (using acridine orange)	95–107.7	[7]
Yogurt, pure milk, flavored milk	Reaction with fuchsin via ion-association complex	Resonance Rayleigh Scattering Spectrometry (@320, 600 nm)	0–10 mg/L	0.028 mg/L	86.9–112.6	[8]
Diluent of Risperidone Injectable Suspension	Dische reaction with diphenylamine	UV–Vis spectrophotometry (@635 nm)	24–146 µg/mL	6 µg/mL	98.5–101.3	Proposed method

¹ RR: Relative Recovery.

The greenness of the method was assessed using the AGSA metric, which evaluates compliance with the 12 principles of Green Analytical Chemistry. The developed procedure achieved an AGSA score of 57.2%, corresponding to a moderate level of environmental sustainability (Figure 7A). The favorable score is primarily attributed to the straightforward sample preparation and limited resource requirements. However, the use of concentrated hydrochloric acid and glacial acetic acid negatively affected the overall greenness rating.

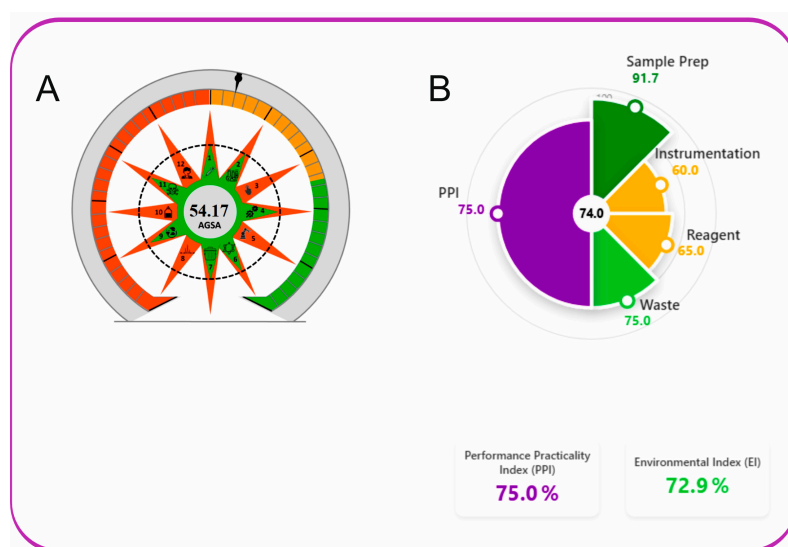


Figure 7. (A) AGSA and (B) EPPI assessment scores of the proposed method for the determination of NaCMC.

The proposed method achieved an environmental index score of 72.9% and a practicality score of 75.0%, indicating a method with low environmental impact and excellent practical

applicability (Figure 7B). These results reflect the simplicity of the analytical procedure, minimal instrumentation requirements, and suitability for routine quality control applications.

Overall, the proposed method can be considered environmentally preferable to many conventional analytical approaches, although opportunities remain for further improvement in reagent selection and waste reduction.

4. Conclusions

A simple, reliable, and economical spectrophotometric method based on the Dische reaction was successfully developed and validated for the determination of NaCMC in the diluent of risperidone injectable suspension. Unlike previously reported methods, which were primarily developed for food and tobacco matrices or rely on fluorescence and resonance Rayleigh scattering techniques, the proposed method is specifically intended for pharmaceutical quality control and requires only widely available UV–Vis instrumentation. The method demonstrated satisfactory specificity, linearity, accuracy, precision, robustness, and solution stability in accordance with ICH guidelines. Successful application to commercial batches confirmed its suitability for routine quality control testing. Furthermore, environmental and practicality assessments indicated acceptable sustainability and excellent applicability in a laboratory setting. Overall, the proposed method provides a practical alternative to more complex and resource-intensive analytical techniques for the routine quantification of NaCMC in pharmaceutical formulations. Although the method was validated using risperidone injectable suspension it has the potential to be applied to other pharmaceutical formulations containing NaCMC, provided that matrix interferences are evaluated and the method is appropriately validated.

To expand the potential use of the assay in non-regulated settings, future research may focus on coupling the developed colorimetric reaction with smartphone-based image or absorbance detection platforms, which have recently emerged as attractive alternatives for portable and low-cost analytical measurements [22,23]. Such integration could further improve the accessibility and field applicability of the proposed methodology while maintaining its simplicity and analytical performance.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/app16167867/s1>. Table S1: Stability data of the standard and test sample solutions.

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