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Research Article

Characterization of Cariprazine Hydrochloride loaded Intranasal Nanoformulation in Simulated Nasal Fluid Using a Validated UV Spectrophotometric Method

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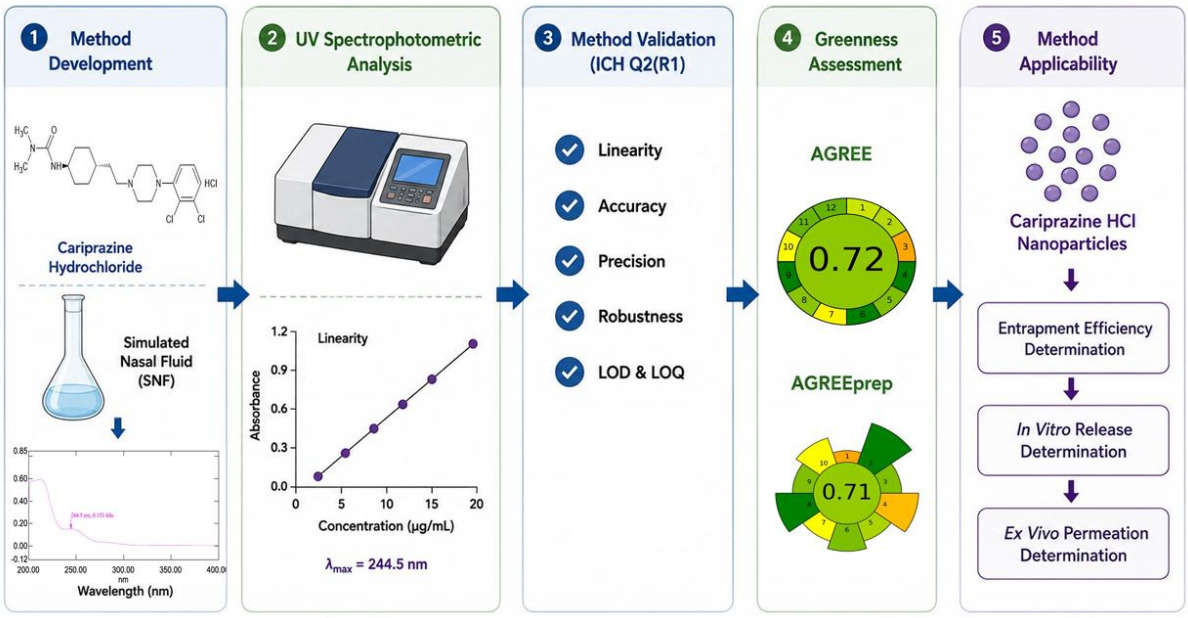
Abstract

Cariprazine hydrochloride belongs to the third generation atypical antipsychotics and possesses a high potential for intranasal drug delivery. Quantitative analysis of the drug-loaded nanoformulations requires validated methods for measuring drug content in simulated nasal fluid (SNF), which is a physiologically relevant medium employed during intranasal formulation development. No validated UV spectrophotometric methods have been described in the literature for estimating the concentration of cariprazine hydrochloride in SNF. In this work, the goal was to establish, validate and show the feasibility of using a UV spectrophotometric method for estimation of the concentration of cariprazine hydrochloride in SNF. The method was developed using SNF, containing 2% (v/v) methanol as the solvent system with measurement of the absorbance at 244.5 nm. Validation of the proposed method was performed following ICH Q2(R1) recommendations by the assessment of linearity, accuracy, precision, sensitivity, and robustness. The validated method was used to conduct the pharmaceutical evaluation of nanostructured lipid carriers loaded with cariprazine hydrochloride by measurement of entrapment efficiency, in vitro drug release and ex vivo permeation. Environmental sustainability of the method was evaluated by AGREE and AGREEprep criteria. The method demonstrated high linearity with correlation coefficient of R^2= 0.9991 within the concentration range of 10-60 µg/ml and regression equation of y = 0.0162x + 0.0028. The obtained percent recovery was 99.90%, and the intra- and inter-day precision %RSD were in the range of 0.072-0.972% and 0.281-1.077%, respectively. Additionally, the method had satisfactory robustness under controlled conditions of analytical variability. The obtained LOD and LOQ values were 1.91 µg/mL and 5.78 µg/mL, respectively. The greenness of the method was confirmed by obtaining AGREE and AGREEprep scores of 0.72 and 0.71, respectively. Besides that, the validated method was successfully used to determine the entrapment efficiency, in vitro drug release and ex vivo permeation of nanostructured lipid carriers of cariprazine hydrochloride, making it a valuable analytical tool for routine intranasal formulation development and quality control.

Keywords: Cariprazine, UV spectrophotometry, method development, method validation, simulated nasal fluid, green analysis

Graphical abstract



1. Introduction

Cariprazine hydrochloride is a 3rd generation atypical antipsychotic drug indicated for schizophrenia and bipolar I disorder. Pharmacologically, it is a partial agonist of the dopamine D3 and D2 receptors with a much higher affinity for the D3 receptor, but also has partial agonistic properties for serotonin 5- HT1A receptors. This special receptor profile makes it an effective drug for reducing both positive and negative symptoms of schizophrenia, and has a relatively lower incidence of extrapyramidal side effects than traditional antipsychotics[1,2]. Cariprazine hydrochloride is trans-N-{4-[2-[4-(2,3-dichlorophenyl)piperazin-1-yl]ethyl}cyclohexyl-N',N'-dimethylurea hydrochloride, with its chemical formula being $C_{21}H_{33}Cl_3N_4O$ and a molecular weight of 463.87 g/mol [3].

Despite its clinical efficacy, oral administration of cariprazine hydrochloride presents several challenges for central nervous system therapy. Oral drug administration is often accompanied by extensive first-pass metabolism and delayed onset of therapeutic levels, and by low bioavailability at the target site as is the case for many antipsychotic medications. Thus, alternative delivery strategies that are able to enhance drug transport to the brain have garnered growing interest. Of these, one method that shows promise is intranasal administration, as it does not go through hepatic first-pass metabolism and can directly access the central nervous system via olfactory/ trigeminal pathways for potentially rapid brain delivery and enhanced therapeutic efficiency [4,5].

For successful development of intranasal formulations, it is necessary to have a reliable analytical method for quantifying drug concentration during formulation optimization. Simulated nasal fluid (SNF) is a physiologically relevant medium to evaluate formulation performance during early development such as determination of drug content, entrapment efficiency, in vitro drug release and in vitro or ex vivo permeation studies. Accordingly, analytical methods used for these investigations should be sufficiently sensitive, and simple, fast and inexpensive enough to allow evaluation of several formulations and sampling times.

While chromatographic methods, such as HPLC and LC-MS/MS, are still reference methods used for trace-level drug quantitation, impurity profiling and bioanalytical applications, they typically require complex sample preparation steps, costly instrumentation, high amounts of organic mobile phases and longer analytical run times [6,7]. The need for such requirements not only adds to the analytical cost, but also increases the amount of solvents used, the energy requirement, and the amount of waste generated in laboratories, making such techniques less practical for routine formulation screening.

UV-visible spectrophotometry, on the other hand, provides a simple and cost-effective method for routine pharmaceutical analysis. Preparation of the samples is typically very simple, involving just a dilution and filtration, and a minimum of internal standards or long extraction processes are required to complete the measurements. This has led to a significant reduction in solvent use, operational cost and turnaround time of analytical results, with acceptable accuracy and precision in the application of formulation development and quality control for analyte concentration in the range of a few micrograms per litre [8–10] . As a result of these benefits, UV spectrophotometry remains one of the most commonly used analysis methods in the early stages of the formulation development of pharmaceuticals[11].

There are several analytical techniques reported for the estimation of cariprazine hydrochloride in pharmaceutical formulations and biological matrices such as UV spectrophotometric and chromatographic. However, these methods have been mainly based on traditional solvents and are not designed to assess the quantification of the drug itself in SNF. Physiological salts and co-solvents can be present in SNF, and may change the spectral properties of the analyte requiring matrix-specific method development and validation. No validated spectrophotometric method using UV has been reported for the estimation of cariprazine hydrochloride in SNF up to date.

In addition to the analytical performance, the environmental aspects of the analytical procedures are also important in pharmaceutical analysis. Green Analytical Chemistry (GAC) aims to develop analytical methods that reduce the quantities of toxic solvents used, waste produced, and/or energy consumption in the analysis without sacrificing the analytical quality. In recent years, analytical methods are increasingly being evaluated quantitatively with the principles of GAC [12,13] using comprehensive assessment tools for greenness, like AGREE or AGREEprep [14,15]. The proposed method was also systematically evaluated for greenness using these metrics.

Thus, the aim of the present study was to develop and validate a simple, rapid, accurate, and eco-friendly UV spectrophotometric method for the quantification of cariprazine hydrochloride in SNF in accordance with the ICH Q2(R1) guideline. In addition to analytical validation, the environmental performance of the developed method was systematically evaluated using AGREE and AGREEprep metrics to assess its greenness. Furthermore, the practical applicability of the validated method was demonstrated through its successful application in the pharmaceutical evaluation of intranasal cariprazine hydrochloride-loaded nanostructured lipid carriers, including entrapment efficiency determination, in vitro drug release, and ex vivo permeation studies, thereby establishing it as a reliable and sustainable analytical tool for routine intranasal formulation development.

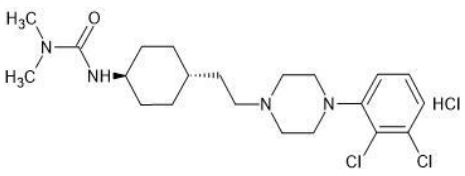


Figure 1. Structure of Cariprazine hydrochloride

2. Materials and Methods

2.1. Materials

Cariprazine HCl was received as a gratis sample from MSN Laboratories pvt. ltd., Telangana, India. Methanol of HPLC grade was procured from Merck (India) lmt. Triethanolamine was procured from Sigma-Aldrich. All the other chemicals and reagents that were used in the study were of analytical grade.

2.2. Method development

2.2.1. Instrumentation

Spectrophotometric analysis was performed using a double beam Shimadzu UV-visible spectrophotometer (model-1900I, Kyoto, Japan) with a path length of 10 mm and a pair of quartz cells was used for analysis.

2.2.2. Preparation of Simulated Nasal Fluid

SNF was prepared according to standard compositions reported for nasal physiological conditions. Briefly, KCl (1.29 mg/ml), NaCl (7.45 mg/ml), $CaCl_2.2H_2O$ (0.32 mg/ml) were accurately weighed and dissolved in 1000 ml of distilled water [16]. Triethanolamine was used to adjust the pH of the solution to 6.75, corresponding to the physiological nasal pH [17].

2.2.3. Selection and optimization of solvent

For intranasal delivery of cariprazine hydrochloride, it is necessary to estimate the drug concentration in SNF. Owing to the drug's limited solubility in SNF, different concentration of methanol in SNF was used to solubilize it. Out of which SNF with 2 % methanol v/v dissolved the drug properly, provided a clear and stable solution for UV spectrophotometric analysis and met all the conditions pertaining to peak quality at observed absorbance maxima.

2.2.4. Preparation of Standard stock solution

1 mg/ml stock solution of cariprazine hydrochloride was made by accurately weighing 10 mg of drug and dissolving in 200 µl of methanol and making up the volume with SNF in 10 ml volumetric flask.

2.2.5. Preparation of working standard solution

From the primary standard stock solution prepared, aliquot of 1ml was transferred and diluted with SNF in 10 ml volumetric flask to obtain a secondary stock solution with concentration of 100 µg/ml.

2.2.6. Preparation of calibration standards

Aliquots from the working standard solution were transferred to 10 ml volumetric flasks and diluted with SNF to achieve concentrations ranging from 10 to 60 µg/ml. Prior to measurement, all samples were filtered through 0.45 µm nylon membrane filter to remove any particulate matter and ensure optical clarity.

2.2.7. Selection of wavelength

Prepared cariprazine hydrochloride solution of 40 µg/ml was scanned between 200 to 400 nm against a drug free SNF-methanol mixture, which served as the blank for baseline correction. The observed λ_{max} was noted as the wavelength where maximum absorption occurred in the prepared solution. Further calibration curve was prepared at this wavelength.

2.3. Analytical validation

2.3.1. Linearity

The linearity of an analytical method is its ability to generate the observed concentrations of the tested samples in proportion to the theoretical concentration of the analyte in the measured samples. Standard sample solutions for cariprazine hydrochloride having concentration ranging from 10 - 60 µg/ml were taken for linearity study. Absorbance was measured against concentration and calibration curve was plotted [18]. Parameters of regression analysis (slope, intercept, correlation coefficient) were obtained by least squares linear regression. Residuals (differences between experimental and predicted absorbance values) were plotted against concentration to validate the appropriateness of the linear relationship and the tests for lack of fit (runs test and deviation from linearity test) were conducted in GraphPad Prism (version 8.0.1). Homoscedasticity of residuals was tested visually while the runs test was used to check the randomness of residuals distribution.

2.3.2. Accuracy

Analytical method is considered accurate if the obtained results are closer to theoretical values. Recovery studies were carried out at three concentration levels, 50% 100% and 150% of the standard concentrations with triplicate analyses at each level to determine accuracy. The test solutions were analysed using the proposed method and the observed concentrations were calculated using the regression equation of the prepared calibration curve and compared against theoretical concentrations [19].

2.3.3. Precision

The precision of the developed method is assessed to evaluate its reliability, reproducibility, and repeatability when the same analytical procedure is repeatedly applied to the same sample under standard experimental conditions. Inter-day and intra-day precision of the samples was assessed at three quality control concentrations (Low, Medium, High), in order to quantify the repeatability of the developed method. Intra-day precision was assessed by analysing 20, 40, and 60 µg/ml cariprazine hydrochloride solutions three times on the same day. Inter-day precision was assessed by testing cariprazine hydrochloride solutions at concentrations of 20, 40, and 60 µg/ml during a period of three consecutive days. Each concentration level (20, 40, and 60 µg/ml) was analysed in triplicate for both intra and inter-day studies. The results are expressed as percent relative standard deviation (%RSD) [20].

2.3.4. Limit of detection (LOD) and limit of quantification (LOQ)

LOD represents the lowest concentration that can be detected with acceptable precision and accuracy, while LOQ represents the lowest concentration that can be

quantified with acceptable precision and accuracy. LOD can be calculated as LOD= 3.3SD/S and LOQ can be calculated as LOQ= 10SD/S. Here SD is the standard deviation of the response [21] estimated either from the y-residuals standard deviation of regression or the standard deviation of the y-intercepts of the regression line [22] and S is the slope of the calibration curve [11]. In the present study, we used standard deviation of the y-intercepts as this approach is widely adopted in UV spectrophotometric method validation studies [23,24].

2.3.5. Robustness

Robustness of the developed method was determined using 40 µg/ml cariprazine hydrochloride concentration. Robustness was estimated by deliberately changing the wavelength by ± 2nm of the λ_{max}. The effect of these changes on the absorbance was observed. Samples were analysed in triplicate and the mean absorbance, standard deviation and % RSD were calculated. Observed concentrations were back calculated from the regression equation to determine the % assay [25].

2.3.6. Repeatability

Repeatability of the method was determined by analysing a standard solution of cariprazine hydrochloride (40 µg/ml) six times and the results are presented as %RSD [26].

2.4. Greenness evaluation of the developed method

The environmental sustainability of the developed UV spectrophotometric method was evaluated using two complementary greenness metric: Analytical greenness (AGREE) and analytical greenness for sample preparation (AGREEprep). Both tools quantitatively assess compliance with the principles of GAC, integrating parameters such as solvent safety, reagent toxicity, energy consumption, waste generation and sample handling complexity [27,28]. The AGREE metric developed by Pareira et al. [14] evaluates analytical methods across 12 principles of Green analytical chemistry and represents the overall greenness score (0-1) through a colour coded circular pictogram, where values closer to 1 indicate higher environmental compatibility. Similarly, AGREEprep introduced in 2022 by the same research group [29], focuses exclusively on the sample preparation stage, applying 10 criteria related to reagent use, process steps, energy demand and waste.

For the current study, both evaluations were conducted using the freely available softwares [14], [29]. The method inputs included the use of methanol as a co solvent at low concentration, minimal sample volume, no derivatization or multi step extraction, short analytical time and low energy consumption. Based on this, the AGREE and AGREEprep scores were calculated and the adherence to sustainable analytical practices was evaluated.

2.5. Preparation of cariprazine hydrochloride loaded nanocarriers

Cariprazine hydrochloride-loaded nanostructured lipid carriers (CP-NLCs) were prepared by the hot high-shear homogenization technique [30]. Briefly, the lipid phase comprising Compritol® E ATO as the solid lipid and oleic acid as the liquid lipid (total lipid concentration: 2% w/v) was heated to 75 ± 2°C until completely melted, and cariprazine hydrochloride was incorporated into the molten lipid phase. Simultaneously, the aqueous phase containing Pluronic® F68 (0.6% w/v) and sodium deoxycholate (0.15% w/v) was heated to the same temperature. The hot aqueous phase was gradually added to the lipid phase and homogenized using a high-shear homogenizer at 15,000 rpm for 10 min to obtain the nanostructured lipid carrier dispersion. The prepared formulation was then allowed to cool down to room temperature to allow for recrystallization of the lipids and formation of nanoparticles, which were then stored at refrigerated temperatures prior to further analysis.

2.6. Characterization of CP-NLCs

The mean particle size, polydispersity index (PDI), and zeta potential of the formulated CP-NLCs were measured using the dynamic light scattering instrument (Horiba SZ-100, Horiba Scientific, Kyoto, Japan). Before conducting the analysis, the suspension of nanoparticles was diluted approximately 100 times with distilled water to avoid multiple scattering. The measurements were carried out at room temperature and three independent replicates were conducted for each measurement.

2.7. Application of the validated UV spectrophotometric method

To demonstrate the practical applicability of the validated UV spectrophotometric method, it was employed for quantitative estimation of cariprazine hydrochloride during the pharmaceutical evaluation of CP-NLCs. It was applied for estimation of entrapment efficiency, in vitro drug release, and ex vivo permeation studies under simulated nasal conditions.

2.7.1. Determination of entrapment efficiency

Entrapment efficiency of CP-NLCs was evaluated by indirect method by measuring the quantity of free drug remaining in the aqueous phase after separation. The prepared formulation sample was centrifuged at 18,000 rpm for 60 min at 4°C to sediment nanoparticles. The supernatant was carefully separated without touching the sediment and appropriately diluted with the optimized analytical medium. The concentration of free cariprazine hydrochloride in the supernatant was measured through the validated UV spectrophotometric method described in this study. All measurements were performed in triplicate (n = 3) using the same nanoparticle batch to evaluate analytical repeatability. The entrapment efficiency was calculated according to the following formula:

$$\text{Entrapment efficiency (\%)} = \frac{\text{Total drug} - \text{Free drug}}{\text{Total drug}} \; x \; 100$$

2.7.2. In vitro drug release study

In vitro release characteristics of CP-NLCs were investigated by using a Franz diffusion cell set up consisting of a dialysis membrane (molecular weight cut off: 12-14 kDa) [31]. The donor chamber contained the CP-

NLC suspension that contained a definite amount of cariprazine hydrochloride, whereas the receptor chamber was filled with SNF at 37 ± 0.5°C temperature with constant magnetic stirring at 50 rpm speed. Samples were withdrawn from the receptor medium at definite time points and the same quantity of fresh SNF medium was replenished into the receptor chamber in order to achieve sink conditions. The samples collected were quantitatively analyzed for drug content using the developed UV spectrophotometry method and the percent drug release was calculated as a function of time.

2.7.3. Ex vivo permeation study

Permeation studies of CP-NLCs were carried out ex vivo using freshly dissected goat nasal mucosa fitted onto the Franz diffusion cell. The freshly dissected mucosa was cleaned extensively with normal saline to wash off any remaining mucus and other particles and then placed carefully between the donor and receiver cells in such a way that the mucosal surface is towards the donor cell. The receiving cell was filled with SNF at 37 ± 0.5°C with continuous magnetic stirring. An accurately measured amount of CP-NLC formulation was added to the donor cell. At definite intervals, 1 ml sample was collected from the receiver cell and immediately replaced with 1 ml of pre-warmed SNF [32]. The amount of permeated drug in each sample was determined using the developed and validated UV spectrophotometric method and the cumulative percentage of drug permeation was calculated.

3. Results and discussion

3.1. Method development and validation

The developed method was validated as per the International Conference on harmonisation ICH Q2(R1) guidelines [33].The maximum absorption of cariprazine hydrochloride solution in SNF was absorbed at 244.5 nm as illustrated in figure 2 and molar absorptivity was found to be 7.51×10^3 $L.mol^{-1}.cm^{-1}$ which has not previously been reported. The observed λ_{max} is consistent with literature reports for cariprazine hydrochloride, where λ_{max} values of 244 nm in water [34] and 252 nm in methanol [35] have been described. An overlay spectrum of the cariprazine hydrochloride working standard concentrations was obtained, demonstrating consistent absorbance characteristics across the analytical range (figure 3).

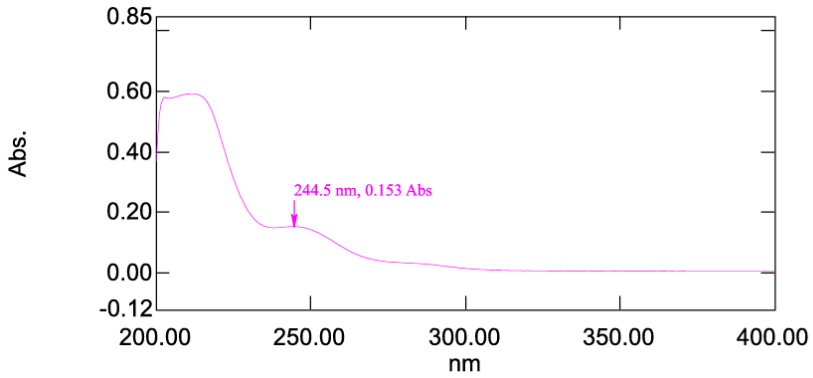


Figure 2. Absorption spectrum of cariprazine hydrochloride in SNF

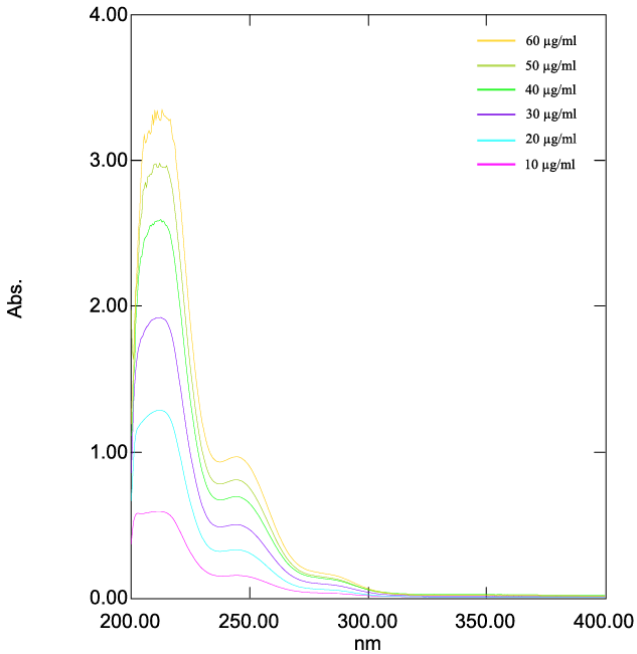


Figure 3. Overlay plot of cariprazine hydrochloride test solutions (10-60 µg/ml) in SNF

3.1.1. Linearity

A calibration curve was prepared by plotting absorbance against concentration over the range of 10 - 60 µg/ml. The data obtained shows that Beer Lambert's law was obeyed in the range of 10 - 60 µg/ml and good linearity was observed with regression equation y = 0.0162x + 0.0028 and R^2 = 0.9991 as shown in figure 4a and table 1.

Residual analysis as shown in figure 4b, indicated random scatter of residuals around zero line without any systematic trend, indicating homoscedastic variance. The runs test (P=0.3) further confirmed the absence of systematic error, and the deviation from linearity was found to be insignificant, confirming the model's adequacy and absence of lack of fit across the studied range.

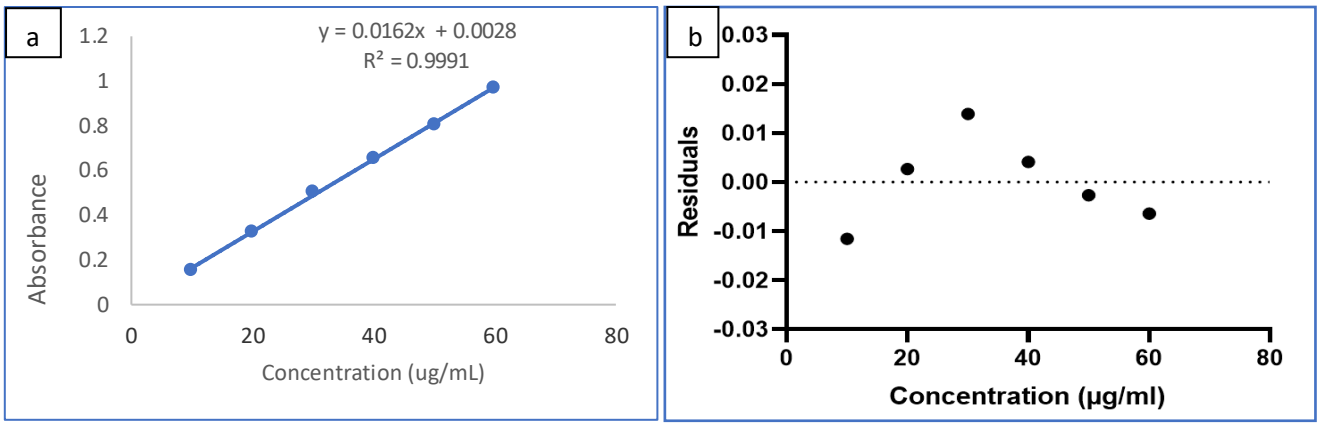


Figure 4. a. Calibration curve of cariprazine hydrochloride in SNF and b. Residual plot demonstrating random distribution of residuals around zero

Table 1. Results of Linearity studies

Parameters	**Value**
Regression equation	y = 0.0162x + 0.0028
Slope(m)	0.0162
Intercept(c)	0.0028
Standard deviation of intercept	0.00936
Regression coefficient R^2	0.9991
Runs test (p-value)	0.3 (Random residuals)
Deviation from linearity	Not significant (No lack of fit)

3.1.2. Accuracy

Recovery studies were performed over three distinct concentration ranges to validate the precision of the developed method. The accuracy of the reanalysed cariprazine hydrochloride test solutions in SNF ranged between 99.64 and 100.17 %, as shown in table 2. According to standard validation guidelines recovery within 98-102% is considered acceptable and all the levels complied with this range, indicating that the developed method is accurate.

Table 2. Results of Accuracy Studies

Concentration level	Theoretical Concentration (µg/ml)	Absorbance* (n = 3)	Calculated Concentration* (µg/ml)	% Accuracy
50 %	20	0.326	19.9788	99.89
100 %	40	0.651	40.0689	100.17
150 %	60	0.970	59.7881	99.64
Average:				99.90

*Average of three estimates

3.1.3. Precision

The %RSD results obtained for intra and interday variability are found to be less than 2% which signifies that the developed method is precise for estimation at level concentrations. % RSD ranged from 0.0720 to 0.9728 for intraday and 0.2818 to 1.0771 for interday precision studies as shown in table 3.

Table 3. Results of Precision Studies

Intraday Precision Studies		
Concentration (µg/ml)	Observed Concentration* (n = 3)	%RSD
20	20.2054	0.9728
40	40.2543	0.4062
60	59.5133	0.0720
Interday Precision Studies		
Concentration (µg/ml)	Observed Concentration* (n = 3)	%RSD
20	20.2741	1.0771
40	40.2612	0.2818
60	59.5476	0.3120

*Average of three estimates

3.1.4. Limit of detection and limit of quantification

LOD and LOQ represents the sensitivity of the developed method and were calculated using standard deviation of the y-intercept and slope of the calibration curve with values determined to be 1.91 µg/ml and 5.78 µg/ml, respectively.

3.1.5. Robustness

To assess the robustness of the developed method, minor variations were introduced in analytical wavelength (242.5, 244.5, and 246.5 nm) as summarized in table 4. The results demonstrated that there were no significant alterations in absorbance or calculated concentration under the tested conditions. The %RSD was below 2% and % assay values ranged between 100.53-101.15 %, indicating the robustness of the developed method.

Table 4. Results of Robustness

Condition	Conc. (µg/ml)	Absorbance*	Standard deviation	%RSD	Observed Concentration*	%Assay
Wavelength 242.5	40	0.6533	0.0015	0.2338	40.2131	100.5327
Wavelength 244.5	40	0.654	0.001	0.1529	40.2543	100.6358
Wavelength 246.5	40	0.6573	0.0015	0.2323	40.4603	101.1509

*Average of three estimates

3.1.6. Repeatability

The repeatability of the developed method was determined by analysing 40 µg/ml cariprazine hydrochloride solution for six times. %RSD was calculated as 0.3120 which is below the acceptance criteria (< 2 %), as shown in table 5.

Table 5. Results of Repeatability

Conc. (µg/ml)	Observed Concentration* (n=6)	Absorbance* (n=6)	Standard deviation	%RSD
40	40.2646	0.6542	0.0020	0.3120

*Average of six estimates

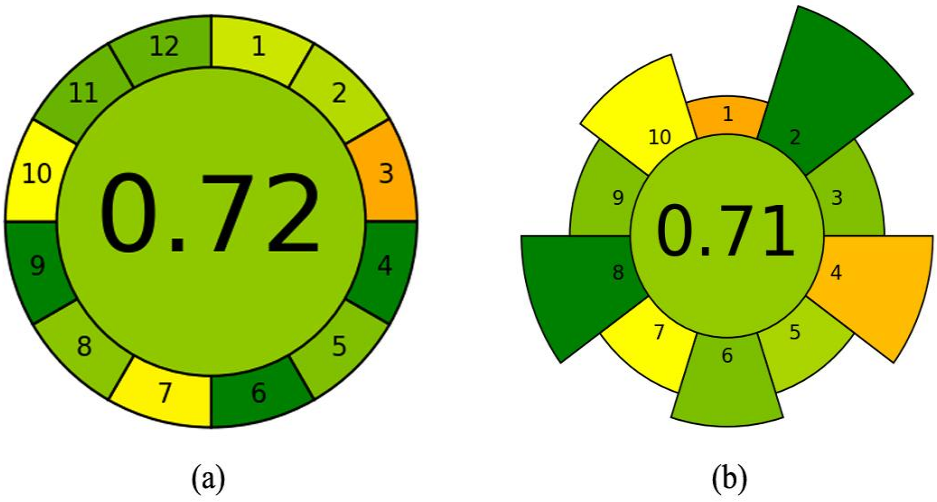
According to the proposed methods the validation parameters were evaluated and all the validated parameters fall within the range. The summary of validated parameters is shown in Table 6.

Table 6. Summary of Validated parameters

Validation parameters	Result
Absorption Maxima	244.5 nm
Linearity Range	10-60 µg/ml
Regression equation	y = 0.0162x + 0.0028
Slope (m)	0.0162
Intercept (c)	0.0028
Regression Coefficient	0.9991
% Accuracy	99.90
Intraday precision	0.072-0.972
Interday precision	0.281-1.077
LOD	1.91 µg/ml
LOQ	5.78 µg/ml

3.2. Greenness Evaluation of the developed method

Environmental sustainability of the proposed UV spectrophotometry method was evaluated by calculating the values of two green analytical chemistry indexes AGREE and AGREEprep, which assess the whole process and sample preparation step, respectively. The pictograms and values are given in Figure 5.



(a) (b)

Figure 5. Greenness assessment pictograms for the developed UV method a. AGREE pictogram b. AGREEprep pictogram

3.2.1. AGREE assessment

The result of the AGREE evaluation is an overall score of 0.72, which means that the suggested analytical procedure shows high level of conformity with the principles of Green Analytical Chemistry (GAC). The good score is mainly due to the simplicity of the UV spectrophotometric method requiring the minimum of equipment, insignificant energy consumption and no derivatization and sample preparation. This method was also evaluated well in terms of criteria on analytical integration, energy efficiency, derivatization elimination, operator safety and replacement of hazardous reagents. Moderate scores were observed in relation to such parameters as in situ analysis, miniaturization, and automation, and generation of wastes has been identified. These indicators have been influenced by the manual preparation of the sample and the application of the small quantity of methanol as the co-solvent prior to the spectrophotometric analysis. On the other hand, the parameter of using the renewable reagents was evaluated as medium due to the fact that methanol, although applied in small quantities, is not considered renewable. Nevertheless, the suggested methodology greatly reduces solvent usage in comparison with the conventional chromatographic techniques.

The intermediate results were obtained in such criteria as direct in situ measurement, miniaturization and automation, and waste generation, which can be attributed to manual sample preparation and the use of methanol as a co-solvent in small amounts prior to the spectrophotometric measurement. Also, the average score was obtained for the criterion on renewable reagents because of the use of methanol, which is not a renewable solvent but is used in small amounts. However, it is necessary to note that the proposed analytical method uses smaller amounts of solvents compared to conventional methods of chromatography.

3.2.2. AGREEprep assessment

Sample preparation greenness was assessed via AGREEprep with the result of 0.71 points, thus demonstrating the environmental friendliness of the proposed sample preparation procedure. Excellent results have been achieved in terms of hazardous substance and energy consumption because of the very small quantity of hazardous substances (methanol in the amount of 0.002 ml of hazardous substance for one analysis), room temperature sample treatment, and low energy extraction method. The performance of the sample preparation procedure in terms of sample throughput, material sustainability, and UV-spectrometric suitability is rather high.

Sample preparation placement, automation, operator safety, and waste generation were given low scores due to the largely manual process of diluting and filtering and the production of aqueous methanol waste post-analysis in a small quantity. While methanol is regarded as a flammable solvent, the low volumes involved and handling procedures employed within normal lab safety protocols reduce any potential dangers posed by this procedure to minimal levels.

The AGREEprep scorecard shows that the proposed sample preparation protocol is simple to use, conserves resources and is environmentally friendly at the same time.

According to the results obtained by AGREE and AGREE prep (with the values of 0.72 and 0.71 respectively), it is possible to affirm that the developed UV spectrophotometry technique for cariprazine hydrochloride is sustainable. In this regard, the technique implies the dilution of the samples and detection without any derivatization, extraction, and application of significant amounts of solvents; therefore, it leads to reduced chemical usage, energy consumption, and waste analysis. Unlike chromatographic methods, which usually involve higher volumes of the organic mobile phase, more complicated equipment and more time, the proposed method provides efficient quantitative results using much fewer resources.

3.3. Characterization of CP-NLCs

The prepared CP-NLCs exhibited a mean particle size of 178.2 ± 3.9 nm, with a PDI of 0.32 ± 0.07 and a zeta potential of −41.1 ± 1.8 mV, indicating the successful preparation of a nanosized lipid carrier system with satisfactory homogeneity and colloidal stability(Figure 6a and 6b). The particle size was within the desirable range for intranasal drug delivery, where nanosized carriers are expected to facilitate efficient interaction with the nasal mucosa and improve drug transport. The PDI value suggested a relatively uniform particle size distribution, while the high negative zeta potential indicated good electrostatic stability, minimizing the likelihood of particle aggregation. Overall, the obtained physicochemical characteristics confirmed the suitability of the prepared CP-NLCs for intranasal delivery.

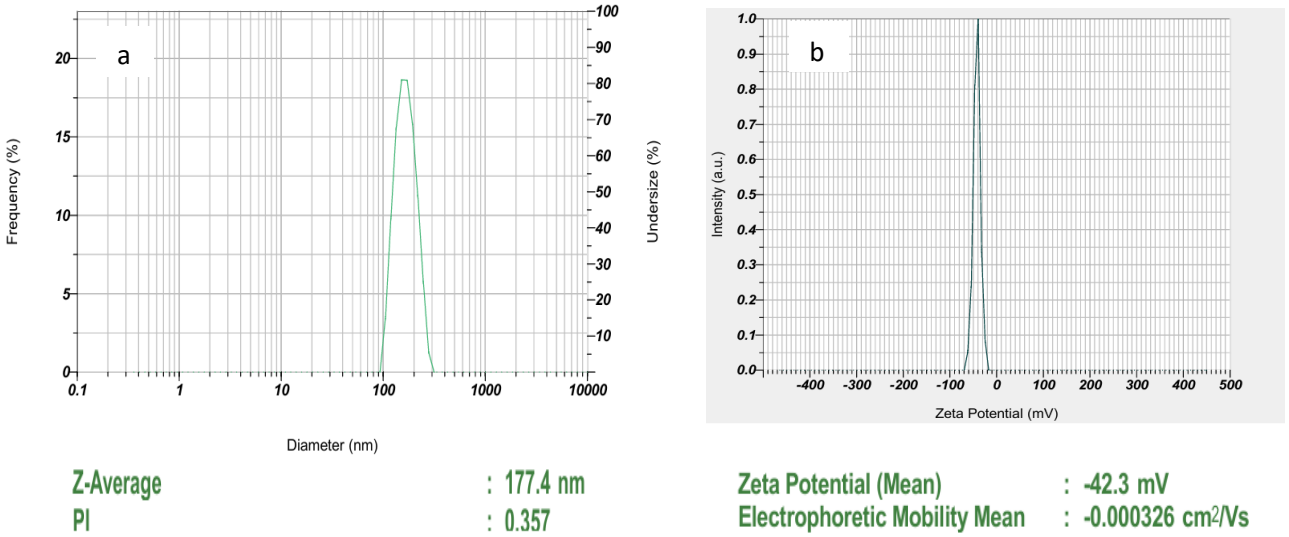


Figure 6. a. Particle size of CP-NLC and b. Zeta potential of CP-NLC

3.4. Application of the validated UV spectrophotometric method

3.4.1. Entrapment efficiency

The validated UV spectrophotometric method was successfully applied for the determination of entrapment efficiency of the prepared CP-NLCs. The formulation exhibited an entrapment efficiency of approximately 71.1 ± 1.07%, indicating satisfactory incorporation of cariprazine hydrochloride within the lipid matrix. The incorporation of the liquid lipid into the solid lipid matrix creates structural imperfections that enhance drug accommodation and minimize drug expulsion during recrystallization, thereby contributing to efficient drug entrapment. Furthermore, the successful quantification of the unentrapped drug using the developed analytical method demonstrated its applicability for routine evaluation of lipid-based nanoformulations, highlighting its potential as a simple and economical alternative for pharmaceutical analysis.

3.4.2. In vitro drug release study

The in vitro release profile of the CP-NLCs demonstrated a biphasic release pattern characterized by an initial burst release followed by sustained drug release over 48 h as shown in Figure 7a. Approximately 11.48% of cariprazine hydrochloride was released within the first 0.5 h, which gradually increased to 49.94% at 6 h and reached 90.24% after 48 h. The initial burst release may be attributed to the diffusion of drug molecules adsorbed or weakly associated with the nanoparticle surface, whereas the subsequent sustained release is primarily governed by diffusion of the entrapped drug through the lipid matrix. Such a release profile is advantageous for intranasal drug delivery, as it may provide an initial therapeutic concentration followed by prolonged drug release, thereby reducing the frequency of administration. The release data showed the best fit to the Korsmeyer–Peppas kinetic model with the highest correlation coefficient of 0.9558, indicating that drug release from the nanostructured lipid carrier was predominantly governed by diffusion through the lipid matrix. Furthermore, the successful quantification of release samples throughout the study using the validated UV spectrophotometric method demonstrated its suitability for routine monitoring of drug release from intranasal lipid-based nanoformulations under simulated nasal conditions.

3.4.3. Ex vivo permeation study

The ex vivo permeation profile of CP-NLCs across goat nasal mucosa demonstrated sustained and enhanced drug permeation over the study period (Figure 7b). The cumulative drug permeation gradually increased from 7.82% at 0.5 h to 44.92% at 12 h, reaching 64.90% after 48 h. This sustained permeation behaviour can be attributed to the lipid matrix of the nanostructured lipid carriers, which facilitates controlled drug diffusion across the nasal mucosa over an extended period. Furthermore, the validated UV spectrophotometric method enabled reliable quantification of permeated drug in SNF, demonstrating its suitability as a simple, economical and effective analytical tool for pharmaceutical evaluation of intranasal nano-formulations.

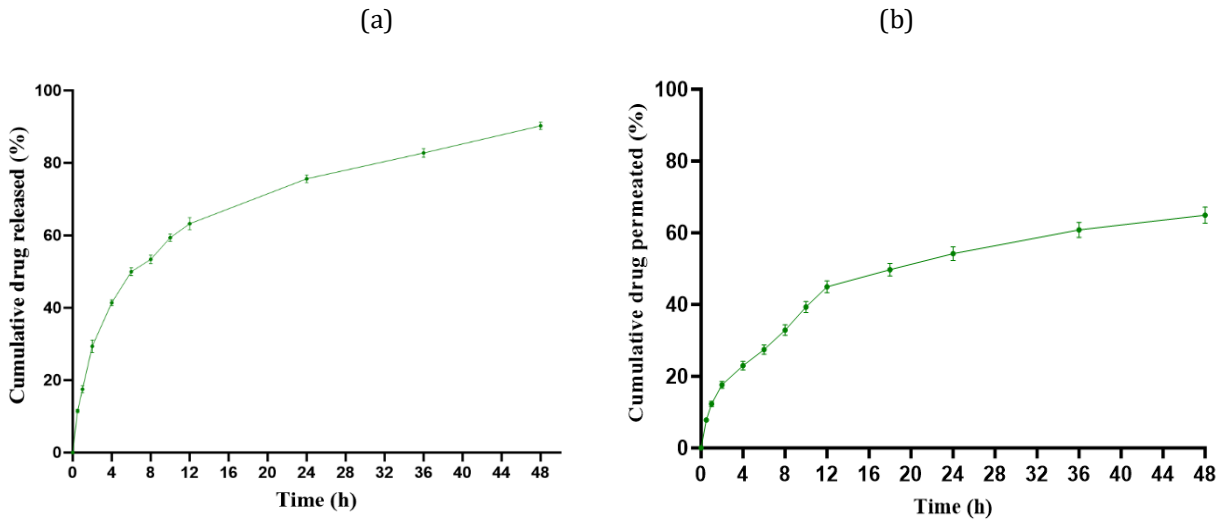


Figure 7. Graphical illustration of a. in-vitro drug release profile and b. ex vivo drug permeation profile of CP-NLCs

4. Conclusion

An efficient, fast, and cost-effective UV spectrophotometric method was successfully developed and validated for cariprazine hydrochloride determination in SNF. The developed method exhibited an impressive level of specificity, linearity, accuracy, precision and robustness, thus meeting the necessary validation criteria according to the ICH Q2(R1) recommendations. In addition, AGREE and AGREEprep analyses have shown that the environment-friendly nature of the developed method due to its low solvent consumption, low energy input, and decreased amount of analytical waste. In addition, the validated method was successfully employed for the pharmaceutical characterization of nanostructured lipid carriers loaded

with cariprazine hydrochloride for the determination of entrapment efficiency, in vitro release, and ex vivo permeation tests. Due to the simplicity, reliability, cost-effectiveness, and green analytical aspects of the proposed method, it can be suitably used for the pharmaceutical quality control and evaluation of intranasal nanoformulations.

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