FORMULATION AND CHARACTERIZATION OF NANOSTRUCTURED LIPID CARRIER INTRANASAL SPRAY OF CARIPRAZINE USING QBD APPROACH

Thesis submitted to

THE KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH, BELAGAVI

(KLE DEEMED UNIVERSITY)

[Declared as Deemed-to-be-University u/s 3 and 12 B of the UGC Act, 1956 vide Govt. of India Notification No.F.9-19/2000-U.3 (A)]
Accredited 'A^{+}' Grade by NAAC (3rd Cycle)
Placed in Category 'A' by MoE(GoI)

For the award of the degree of

DOCTOR OF PHILOSOPHY
IN

THE FACULTY OF PHARMACY

By

PALLAVI RAOSAHEB CHIPRIKAR M.Pharm.

(Reg No: KLEU/Ph.D./20-21 /DO1220047)

Under the Guidance of

PROF (Dr). VINAYAK MASTIHOLIMATH Ph.D

Department of Pharmaceutical Quality Assurance

KLE College of Pharmacy,
KLE Academy of Higher Education and Research, Belagavi- 590010.

JANUARY - 2024

UNDERTAKING

I, **Mrs. Pallavi R. Chiprikar** hereby declare that the information and the data mentioned in my thesis entitled **"Formulation and Characterization of Nanostructured lipid carrier Intranasal Spray of Cariprazine using QbD approach"** belongs to me and is original.

I am aware of definition of plagiarism as detailed below:

- An act or instance of using or closely imitating the language and thoughts of another author without authorization and the representation of that author's work as one's own, as by not crediting the original author.
- A piece of writing or other work reflecting such unauthorized use or imitation.
- The deliberate or reckless representation of another's words, thoughts or ideas as one's own without attribution in connection with submission of academic work, whether graded or otherwise.

I hereby declare that the thesis prepared by me is original-one and does not involve plagiarism anywhere. In case at a later stage it is found that I have indulged in plagiarism, then I am solely responsible for the same and the Institution is at liberty to take any disciplinary action against me including cancellation of dissertation or any other penalties imposed by the University.

Date:

Place: Belagavi

Mrs. Pallavi R. Chiprikar
Full time PhD Research Scholar
Reg. No: DO1220047
KAHER, Belagavi- 590010

Ref. No. KAHER/AA/23-24/D-355 2nd February 2024

Madam,

The soft copy of Ph.D. research thesis of **Mrs. Pallavi R. Chiprikar, KLE College of Pharmacy, Faculty of Pharmacy, Belagavi** has been submitted for anti-plagiarism check at the office of the undersigned through "Turn-it-in" package. The scan has been carried out and the scanned output reveals a match percentage of **7%** which is within the acceptable limit of 10%.

To obtain the comprehensive report of the plagiarism test, research scholar can send a mail to **diracademic@kledeemeduniversity.edu.in** along with the Registration Number, Name of the Scholar, Name of Guide/Co-guide and title of the thesis.

DIRECTOR ACADEMIC AFFAIRS Deemed-to-be University

Dr.(Mrs.) Roopa M. Bellad
Director, Academic Affairs

To,

Mrs. Pallavi R Chiprikar
Full-Time Ph.D. Scholar,
2020-21 Batch, Faculty of Pharmacy,
KLE College of Pharmacy,
Belagavi.

Cc to :

1. The Principal, KLE College of Pharmacy, Belagavi
2. Dr. V.S. Mastiholimath, Professor of Pharmaceutics, KLE College of Pharmacy, Belagavi -Guide

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH,

(KLE DEEMED UNIVERSITY)

[Declared as Deemed-to-be-University u/s 3 and 12 B of the UGC Act, 1956 vide Govt. of India Notification No.F.9-19/2000-U.3(A)]

Accredited 'A^{+}' Grade by NAAC (3rd Cycle)
Placed in Category 'A' by MoE (GoI)
BELAGAVI

COPYRIGHT DECLARATION

We hereby declare that **KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH, BELAGAVI, KARNATAKA,** *shall have the rights to preserve, use and disseminate this thesis in print or electronic format for academic/research purpose.*

Signature

Mrs. Pallavi R. Chiprikar
Full time PhD Research Scholar
Reg. No: DO1220047
KAHER, Belagavi- 590010

Signature

Prof (Dr). Vinayak Mastiholimath
Dept of Pharmaceutical Quality Assurance
KLE College of Pharmacy,
KAHER, Belagavi-590010.

Place: Belagavi

Date:

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH,

(KLE DEEMED UNIVERSITY)

Declared as Deemed-to-be-University u/s 3 and 12 B of the UGC Act, 1956 vide Govt. of India Notification No.F.9-19/2

\000-U.3 (A)

Accredited 'A^{+}' Grade by NAAC (3rd Cycle)
Placed in Category 'A' by MoE (GoI)
BELAGAVI

DECLARATION

I hereby declare that the thesis entitled **"FORMULATION AND CHARACTERIZATION OF NANOSTRUCTURED LIPID CARRIER INTRANASAL SPRAY OF CARIPRAZINE USING QBD APPROACH"** *is a bonafide and original research carried out by me under the guidance of* **Prof. (Dr.) Vinayak Mastiholimath,** *Department of Pharmaceutical Quality Assurance, KLE College of Pharmacy, Belagavi. The thesis or any part thereof has not formed the basis for the award of any degree/fellowship or similar title to any candidate of any University.*

Place: Belagavi

Date:

Signature

Mrs. Pallavi R. Chiprikar
Full time PhD Research Scholar
Reg. No: DO1220047
KAHER, Belagavi- 590010

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH,

(KLE DEEMED UNIVERSITY)

[Declared as Deemed-to-be-University u/s 3 and 12 B of the UGC Act, 1956 vide Govt. of India Notification No.F.9-19/2000-U.3(A)]

Accredited 'A^{+}' Grade by NAAC (3rd Cycle)
Placed in Category 'A' by MoE (GoI)
BELAGAVI

CERTIFICATE

This is to certify that the thesis entitled **"FORMULATION AND CHARACTERIZATION OF NANOSTRUCTURED LIPID CARRIER INTRANASAL SPRAY OF CARIPRAZINE USING QBD APPROACH"** *is a bonafide and genuine research carried out by* **Mrs. Pallavi Chiprikar** *under the guidance of* **Prof. (Dr.) Vinayak Mastiholimath**, *Department of Pharmaceutical Quality Assurance, KLE College of Pharmacy, Belagavi.*

Place: Belagavi

Date:

Signature

Prof. (Dr.) M. S. Ganachari

Dean, Faculty of Pharmacy

KAHER, Belagavi- 590010.

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH,

(KLE DEEMED UNIVERSITY)

[Declared as Deemed-to-be-University u/s 3 and 12 B of the UGC Act, 1956 vide Govt. of India Notification No.F.9-19/2000-U.3(A)]

Accredited 'A^{+}' Grade by NAAC (3rd Cycle)
Placed in Category 'A' by MoE (GoI)
BELAGAVI

CERTIFICATE

This is to certify that the thesis entitled **"FORMULATION AND CHARACTERIZATION OF NANOSTRUCTURED LIPID CARRIER INTRANASAL SPRAY OF CARIPRAZINE USING QBD APPROACH"** *is a bonafide and genuine research carried out by* **Mrs. Pallavi Chiprikar** *under the guidance of* **Prof. (Dr.) Vinayak Mastiholimath**, *Department of Pharmaceutical Quality Assurance, KLE College of Pharmacy, Belagavi.*

Place: Belagavi

Date:

Prof (Dr). Sunil Jalalpure
Principal,
KLE College of Pharmacy,
KAHER, Belagavi-590010.

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH,

(KLE DEEMED UNIVERSITY)

[Declared as Deemed-to-be-University u/s 3 and 12 B of the UGC Act, 1956 vide Govt. of India Notification No.F.9-19/2000-U.3 (A)]

(Accredited 'A^{+}' Grade by NAAC) (3rd Cycle)
[Placed in Category 'A' by MoE (GoI)]
BELAGAVI

CERTIFICATE

This is to certify that the thesis entitled **"FORMULATION AND CHARACTERIZATION OF NANOSTRUCTURED LIPID CARRIER INTRANASAL SPRAY OF CARIPRAZINE USING QBD APPROACH"** *is a bonafide record of original research carried out by* **Mrs. Pallavi R. Chiprikar** *for the award of degree of* **DOCTOR OF PHILOSOPHY IN FACULTY OF PHARMACY** *under my supervision and guidance.*

Signature

Prof. (Dr.) Vinayak Mastiholimath
Dept. of Pharmaceutical Quality Assurance
KLE College of Pharmacy,
KAHER, Belagavi- 590010

Place: Belagavi

Date:

ACKNOWLEDGMENT

I would like to express my sincere gratitude to the individuals and organizations who have played a significant role in the completion of this thesis. Their support, guidance, and encouragement have been invaluable throughout this academic journey.

Firstly, I would like to heartfelt thanks to to my research guide ***Prof. Dr. Vinayak Mastiholimath.*** *I am sincerely grateful for the guidance, mentorship, and constructive feedback provided throughout the entire research process. His encouragement has been instrumental in shaping the direction of this thesis.*

I am deeply indebted to our Honorable former Vice Chancellor, ***Prof (Dr.) Vivek Saoji,*** *and respected Vice Chancellor* ***Prof. (Dr). Nitin Gangane and Prof. (Dr.) M. S. Ganachari,*** *Dean, Faculty of Pharmacy for giving me an opportunity to join and complete Ph.D. course as Full Time research Scholar at KLE College of Pharmacy, Belagavi. I express my gratitude to Director of Acadamic affairs* ***Dr, Roopa Bellad,*** *KLE Acadamy of Higher Education and Research for their support and co-operation.*

I would like to thank Principal ***Prof. (Dr). Sunil. S. Jalalpure****, KLE College of Pharmacy, Belagavi, for providing invigorative environment to pursue this research work with great ease.*

I would also like to thank Vice-principal ***Prof. (Dr). M. B. Patil****, KLE College of Pharmacy, Belagavi, for constant help and support.*

My special thanks goes out to ***Dr. V. S. Mannur****, Head of the Pharmaceutical Quality Assurance Department at the KLE College of Pharmacy in Belagavi who helped and cooperated during the research.*

I owe my special thanks to ***Mr. Prakash R. Biradar****, Assistant Professor, Department of Pharmacology, KLE College of Pharmacy, Belagavi, for helping me with animal studies.*

I express my deep gratitude to all my respected staff members especially ***Mrs. Nisha Shikroli, Shailendra Suryawanshi, Dr. Bhaskar Kuranagi, Dr. Archana Patil****, and the entire teaching and non-teaching staff of KLE'S College of Pharmacy for providing their timely help.*

I appreciate the resources and facilities provided by KAHER University, Belagavi. I would like to acknowledge the financial support (Scholarship) received from KAHER University, which played a crucial role in facilitating my research.

I wanted to take a moment to express my sincere gratitude to ***Prof. D. D. Chougule and Dr. C. S. Magdum*** *who uplifted me for the personal challenges in my life. Your support, and belief in me have been invaluable, and it has made a significant impact on my life.*

I would like to thank my friends Pradnya Wadekar, Sudhir Patil, Pranita Chougule, Jyoti Patil Mam, Priya Patil, my student Sagar Salave and colleagues for their intellectual discussions, and the shared moments of both challenge and success. Your friendship has added a meaningful dimension to my academic journey.

I am thankful to my PhD colleagues Anand Ammange, Yeswanth Raja, Amruta Balekundri, Sowmya Spoorthi who helped me and supported me since the early days of my research work.

I would like to express my gratitude to my parents R. D. Chiprikar and Pushpalata Chiprikar for their enormous support, encouragement, love and prayers. I extend my gratitude to my beloved sisters Pranoti and Priyanka for their support. Their belief in my abilities has been a constant source of motivation.

I remember with thanks the immense emotional strength and encouragement received from my husband Prasanna M and the genuine warmth and enthusiasm of my daughter Amolika and son Pranmya, which sustained me through this hectic work. My special thanks to Ballawwa ajji who look after my kids whenever I was in college.

My special thanks to MSN Laboratories Private Limited, Hyderabad, Telangana, India for providing gift sample of Cariprazine Hydrochloride for my research work.

I am truly thankful to all those who have been a part of this academic journey. Your contributions have enriched my learning experience, and I am grateful for the opportunity to undertake this research.

Date: **Mrs. Pallavi Chiprikar**

TABLE OF CONTENTS

Sl. No.	Particulars	Page No.
1.	**Introduction**	1-18
1.1	Background	
1.2	Review of Literature	
1.3	Justification	
1.4	Objective and plane of work	
2.	**Material and Methods**	19-32
3.	**Results and Discussion**	33-64
4.	**Summary**	65-66
5.	**Conclusion**	67-68
6.	**Bibliography**	69-80
8.	**Annexure**	81
	a. Animal Ethical Clearance letter	
	b. Publications and poster presentation	

LIST OF ABBREVIATIONS

ANOVA	:	Analysis of variance
BBB	:	Blood-brain barrier
BBD	:	Box Behnken Design
Cm	:	Centimeter
cP	:	Centipoises
CPCSEA	:	Committee for the Purpose of Control and Supervision of Experiments on Animals
CPZ	:	Cariprazine
DSC	:	Differential scanning calorimetery
DCAR	:	Desmethyl Cariprazine
DDCAR	:	Didesmethyl cariprazine
EE	:	Entrapment Efficiency
EPS	:	Extrapyramidal symptoms
FT-IR	:	Fourier transform infrared spectroscopy
g	:	Gram
GIT	:	Gastrointestinal tract
GMS	:	Glyceryl monostearate
h	:	Hour
HCl	:	Hydrochloride
HPLC	:	High Performance Liquid Chromatography
HPTLC	:	High Performance Thin Layer Liquid Chromatography
IAEC	:	Institutional animal ethics Committee
ICH	:	International Council for Harmonization
IPA	:	Isopropyl Alcohol
kg	:	Kilogram
LL	:	Liquid Lipid
LOD	:	Limit of detection
LOQ	:	Limit of quantification
min	:	Minute
mg	:	Milligram
ml	:	Mililiter
MRT	:	Mean residence time

Nm	:	Nanometer
NLC	:	Nanostructured lipid carrier
P	:	Probability
PB	:	Phosphate buffer
PDI	:	Polydispersity index
pH	:	Power of Hydrogen
PDA	:	Photo diode aaray {also known as DAD}
r^2	:	Correlation Coefficient
RH	:	Relative Humidity
Rpm	:	Revolution per minute
RP-HPLC	:	Reverse phase High Performance Liquid Chromatography
RSD	:	Relative standard deviation
SD	:	Standard deviation
SL	:	Solid Lipid
TEM	:	Transmission electron microscopy
USFDA	:	United states Food and Drug Administartion
UV	:	ultraviolet
v/v	:	Volume by volume
w/v	:	Weight by weight
w/w	:	Weight by weight
ZP	:	Zeta potential
µg	:	Microgram
µm	:	micrometer
°C	:	Degree centigrade

LIST OF TABLES

Sl. No.	Particulars	Page No.
1	List of instruments	19
2	List of chemicals	20
3	Elements of the QTPP & CQA for development of the CPZ-NLC	24
4	Independent and dependent variable Levels in BBD for CPZ-NLC preparation	27
5	Final Chromatographic Conditions	35
6	Observed responses in BBD design	42
7	Summary of Regression Analysis	45
8	Optimized Formula	46
9	Pharmacokinetic parameters data of CPZ (Oral, Intranasal, CPZ NLC Intranasal)	60
10	Short term Stability study of optimized formulation F6 at room temperature (25°C/60%RH)	62
11	Stability study at accelerated condition (40°C/75%RH)	62
12	Nasal spray evaluation results	64

LIST OF FIGURES

Sl. No.	Particulars	Page No.
1	Structure of Cariprazine Hydrochloride	2
2	Pharmacokinetics of Cariprazine	4
3	Components of nanostructured lipid carriers (NLC): drug, surfactant, liquid lipid (oil), and solid lipid	7
4	Fishbone Diagram for the development of CPZ-NLC formulation	11
5	QbD approach	24
6	Cariprazine hydrochloride standard UV spectrum	33
7	Overlay spectrum of cariprazine hydrochloride	34
8	Cariprazine hydrochloride linearity graph	34
9	HPLC chromatogram for CPZ	35
10	Calibration curve of CPZ by HPLC	36
11	Linearity overlay chromatogram of cariprazine hydrochloride	36
12	A schematic diagram for the HP-TLC instrument along with method development for determination of CPZ	37
13	CPZ Spectra	37
14	CPZ Typical chromatogram	38
15	CPZ NLC formulation at 253 nm	38
16	Developed plate of linearity	39
17	Linear regression of CPZ standards	39
18	Linearity chromatograms of CPZ (1, 2, 3, 4,5 µL/band TRACK 1-5) in 3D view	40

19	Drug solubility in various solid lipids	40
20	Drug solubility in different liquid lipid and surfactant	41
21	The flowchart of the preparation process of CPZ-NLC	41
22	3D Response surface plot for the effect of independent variables on Particle size	43
23	3D Response surface plot for the effect of independent variables on entrapment efficiency	44
24	Overlay plot (Design space)	46
25	Particle size distribution (A) and ZP (B) of optimized formulation	48
26	SEM image of optimized CPZ loaded formulation	49
27	TEM image of optimized CPZ loaded formulation	49
28	DSC thermogram of (A) Cariprazine hydrochloride, (B) Physical Mixture, (C) GMS and CPZ Mixture, (D) CPZ NLCs formulation, (E) Overlay	51
29	FTIR spectra of (A) CPZ, (B) Physical Mixture, (C) CPZ-NLC Formulation and (D) Overlay	53
30	In vitro drug release of F2, F6, F8 batches	54
31	Ex vivo permeation study by sheep nasal mucosa	56
32	Histopathology section of nasal mucosa treated with (A)CPZ NLCs formulation, (B)IPA, and (C)Phosphate Buffer.	57
33	Brain-drug concentration-time profile of CPZ by various route	61
34	(A) Intranasal administration of CPZ NLCs formulation (B) Brain of rat	61

ABSTRACT

Background

Schizophrenia is a complex, long-lasting mental health condition marked by hallucinations, delusions, disorganised speech or behaviour, and impaired cognitive function. Around 24 million people, or 1 in 300 persons (0.32%), globally suffer with schizophrenia.

Cariprazine hydrochloride (CPZ) is a new atypical antipsychotic used to treat schizophrenia. CPZ is a BCS class II drug having low aqueous solubility (0.1159 mg/L). It exhibits oral bioavailability 52%. CPZ has a moderate first-pass effect. Conventional dosage forms of CPZ exhibits adverse effects like insomnia, akathisia, nausea, hyperprolactinemia, EPS, headache, dizziness, tremors, and gastrointestinal disturbances. Many antipsychotics are not suited for oral administration from a pharmacokinetic point of view due to limited bioavailability brought on by gastrointestinal enzymatic degradation, hepatic first-pass effects, and/or gastrointestinal adverse effects. Additionally, due to a lack of aqueous solubility and unfavourable physical/chemical or toxicological qualities, not all medications have injectable drug formulations available. Intranasal delivery of nano lipid carriers enhanced the bioavailability of drug molecules by obviating their first-pass metabolism and delivers them directly to the brain.

Objectives

The present study was an attempt to systemically deliver Cariprazine HCl, a schizophrenic drug via intranasal route using nanostructured lipid carrier.

Methodology

The CPZ loaded NLCs were developed using Box Behnken design and developed formulations were monitered for improving CPZ bioavailability. NLCs loaded CPZ was

formulated by melt emulsification ultrasonication method. NLCs is a blend of solid lipid, liquid lipid and surfactant to encapsulate water-insoluble drugs.

Results

The optimized CPZ- NLC formulation shows entrapment efficiency of 96.1±0.57 %, particle size of 173.3±0.6 nm with polydispersity index 0.30 and zeta potential of 6.22 mV. *In vitro* study showed drug release of 96.11± 2 % at the end of 30 min. An *Ex Vivo* permeability study revealed that, after 35 minutes, 75.83% of the CPZ had permeated. Experiments on nasal ciliotoxicity demonstrated that all of the excipients utilized in the formulation of CPZ-loaded NLCs were safe enough to be delivered through the nasal route. *In Vivo* study showed that concentration of drug that reaches the brain after intranasal administration was much higher as compared with oral administration.

Conclusion

The findings show that nanostructured lipid carrier is novel and potential drug delivery approach for the intranasal administration of Cariprazine hydrochloride in the treatment of schizophrenia.

Keywords

Nanostructured lipid carrier; Cariprazine; Schizophrenia; Intranasal; Box Behnken design; Brain targeting; Solid lipid; Liquid lipid.

INTRODUCTION

1.1 BACKGROUND

Schizophrenia:

The severe mental disorder known as Schizophrenia has an impacts a person's feelings, opinions, and actions. Individuals with schizophrenia may experience a profound detachment from reality, creating distress not only for themselves but also for those in their immediate circle (1). Originally referred to as "dementia praecox" by Kraepelin (1896), and subsequently termed "schizophrenia" by Bleuler (1911)(2). The condition is defined by disturbances in cognition, perception, and a blunting of affect. A person with an active condition may encounter periods of time when they are unable to differentiate between genuine and imaginary sensations. While the intensity, length, and frequency of symptoms might vary, as is the case with schizophrenia typically experience a decrease in severe psychotic symptoms as they older(3).

There are primary categories for symptoms(3)

Positive symptoms encompass exaggerated or distorted perceptions, beliefs, and behaviors in individuals with conditions like schizophrenia. These may manifest as hallucinations, such as hearing voices or seeing nonexistent objects, as well as instances of paranoia.

Negative symptoms include a decrease or loss of the ability to communicate, express emotions, make decisions, or have fun.

Disorganized symptoms include difficulty in thinking and speaking clearly, odd behavior or unusual gestures, abnormal movements, and difficulty in applying reasoning or acting strangely.

Research shows there are multiple causes of schizophrenia. The interaction of genes and environmental factors is believed to be the cause of schizophrenia. The development and progression of schizophrenia can be influenced by psychosocial factors. Excessive cannabis use is associated with an elevated risk of developing this condition (4).

The first line of treatment for symptoms of an acute schizophrenia episode is typically antipsychotics. They work by blocking dopamine or other substance's effect on the brain. Antipsychotics usually take a few hours to start reducing emotions of anger or anxiety, but it may take days or weeks to start reducing other symptoms like hallucinations or delusional thinking (5) (6).

Cariprazine hydrochloride

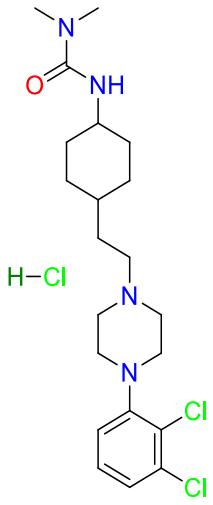


Figure 1: Structure of Cariprazine Hydrochloride

IUPAC name(7)

3-[4-[2-[4-(2,3-dichlorophenyl)piperazin-1-yl]ethyl]cyclohexyl]-1,1-dimethylurea

Molecular formula C21H32Cl2N4O

Molecular weight 427.4

Log P 4.98

pKa1 = 7.91

pKa2 = 15.48

Solubility- Soluble in methanol, DMSO. Water-insoluble (BCS class II drug)

Half-life ~2-4 days

Dose 1.5 mg (maximum tolerated dose per day 6 mg)

Vraylar is a branded medication that falls under the category of atypical antipsychotics, prescribed to control bipolar disorder and schizophrenia symptoms. Cariprazine is the active ingredient in Vraylar, contributing to its therapeutic effects in addressing these mental health conditions. In 2015, Permission to use the drug was given by the Food and Drug Administration (FDA) of the United States in treating schizophrenia among adults. Following that, in 2017, the FDA broadened its approval to include the acute treatment of adult bipolar I disorder-related manic or mixed episodes.

One of the newest “atypical” second generation antipsychotics, or dopamine serotonin partial agonist, is Cariprazine. Originally, the word "atypical" was used to define antipsychotics with a lower or negligible risk of producing EPS (8). Cariprazine belongs to the same pharmacological class as brexpiprazole and aripiprazole as both are partial agonists of dopamine and serotonin. More precisely, Cariprazine exhibits a different profile from both brexpiprazole and aripiprazole, binding with high potency dopamine D3 and D2 receptors as well as 5HT2B serotonin receptors, and moderate potency to the serotonin receptors 5HT1A and 5HT2A (9,10). Didesmethyl and desmethyl cariprazine (DDCAR), its two principal active metabolites, are equally in charge of the overall therapeutic action and are pharmacologically equivalent to cariprazine(8).

CYP3A4 and CYP2D6 metabolises cariprazine to DCAR and DDCAR to a greater or lesser degree. (8). Most common adverse events that have been reported were akathisia, EPS, headache, dizziness, tremors, and gastrointestinal disturbances(11,12).

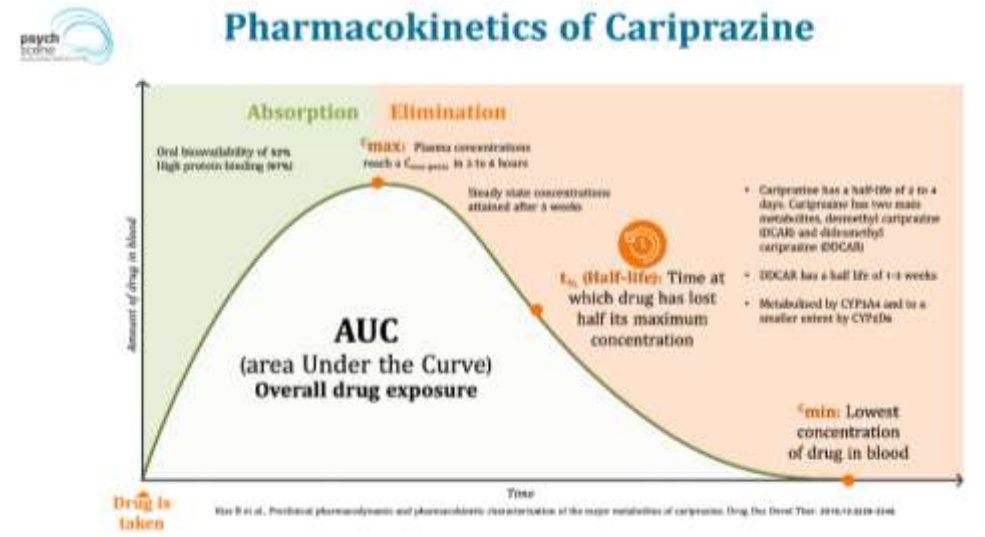


Figure 2: Pharmacokinetics of Cariprazine(12)

Nasal drug delivery:

Nasal drug delivery has a long history that begins with the topical administration of medications meant for localized effects. In the Indian Ayurvedic system, nasal therapy also known as “Nasya Karma”. One major benefit of administering drugs via the nose is that the drug is quickly absorbed because of the physical conditions of the nose, such as the mucosa's good blood circulation, which produces a local effect quickly. Rapid local absorption helps to prevent unintended drug distribution throughout the body and minimizes the side effects of the active ingredient. Nasal mucosa has been seen to getting attention recently as a potential alternative for achieving better and faster drug absorption. The administration of drugs through the nasal route, taking advantage of the high permeability of the nasal mucosa, has a historical legacy dating back thousands of years. The nasal mucosa has been recognized as a therapeutically possible alternative(13). When compared to oral administration, nasal administration offers

numerous benefits, including a high rate of absorption, minimum drug degradation, and a quick onset of action. By utilizing the olfactory nerve pathways to cross the blood-brain barrier, it permits drug delivery from the nasal passage to the brain. It has great patient compliance, and self-administration as compared to intravenous administration(14).

Advantages of nasal drug delivery(15)

1. Quick onset of action and rapid drug absorption.
2. Rapid drug absorption via highly vascularized mucosa.
3. Absorption enhancers and other methods can be used to increase the bioavailability of drug.
4. As an alternative to the parenteral route, the nasal route can be used for proteins and peptides.
5. Large nasal mucosal surface area for absorption.
6. First pass metabolism is absent.
7. Non-invasive and Ease of administration.
8. Minimal side effects.
9. Self-administration.
10. Improved convenience and compliance.

Limitation(15)

1. The safety of absorption enhancers employed in nasal drug delivery systems remains uncertain in terms of their potential toxicity.
2. Nasal irritation is a potential concern.
3. The nasal cavity has a smaller surface area for absorption compared to the gastrointestinal tract (GIT).

Various dosage forms used for nasal drug delivery

1. Nasal powders
2. Nasal gels
3. Nasal sprays
4. Nasal drops

Nanostructured lipid carrier:

A mixture of liquid and solid lipids makes up a formulation known as a nanostructured lipid carrier (NLC). Because NLC is simple to prepare, can be scaled up easily, is non-toxic, has improved targeting efficiency, and can be delivered to a specific site by a variety of administration routes, it has found extensive use in pharmaceutical and cosmetic industries(16). The first class is the imperfect kind, where different lipid structures are combined with both liquid and solid fats (oil). Because of an improper lipid matrix structure that creates a space between the triglyceride fatty acid chains in the crystal, drugs are more able to pass through the matrix. As a result of lacking a crystalline structure, the second class of NLC's known as the amorphous type prevents the expulsion of loaded drugs. This type of NLC is called the formless type (non-crystalline matrix). The drug is more soluble in liquid lipid than in solid lipid known as multiple type. This type of NLC resembles w/o/w emulsions. A larger percentage of oils can be employed in multiple emulsion NLC. Surfactant solutions ranging from 0.5% to 5% help in stabilizing the system.(16,17)

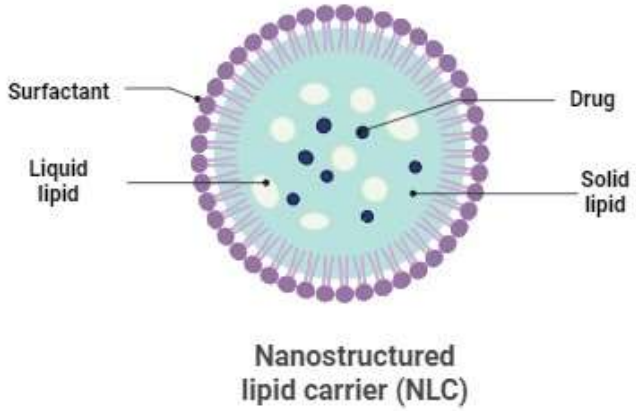


Figure 3: Components of nanostructured lipid carriers (NLC): drug, surfactant, liquid lipid (oil), and solid lipid (Created with Biorender.com)

Advantages of NLC's(18)

1. Improved physical stability.
2. Targeted drug release and controlled particle size.
3. High entrapment of lipophilic drug and feasibility of carrying both lipophilic and hydrophilic drug.
4. Superior drug loading.
5. As it is water-based method, organic solvents can be avoided.
6. Most of lipids are biocompatible and biodegradable.

Brain targeting with NLCs

Drug distribution to the brain is limited by the blood-brain barrier (BBB) therefore, strategies to get beyond the body's defenses are essential. The following features make NLCs a promising strategy for brain targeting (19).

1. Nano size- Due to their nanoscale size, NLCs can be readily internalized and transported via the brain's microvasculature using the endocytosis and transcytosis transport mechanisms.

2. Lipid solubility- Nearly all drugs for the brain are small molecules soluble in lipids. They can traverse the blood-brain barrier (BBB) through free diffusion facilitated by lipids, enabling the delivery of even water-soluble drugs to the brain.
3. Improving the bioavailability and preventing the degradation of labile drugs - It has been shown that NLCs greatly increase the encapsulation efficiency of hydrophilic and hydrophobic labile drugs.
4. Active targeting: The main advantages of active targeting are less dose-related adverse effects and greater drug retention in brain tissue. Active targeting involves modifying carriers, such as ligands, proteins, or receptors, to enhance its capacity to pass through the blood-brain barrier.

Methods of fabrication of NLCs

Nanostructured lipid carriers (NLCs) are typically prepared by emulsifying the aqueous phase, containing surfactants or emulsifiers, with the lipophilic phase, consisting of a combination of lipids, both liquid and solid.

There are several methods used for the preparation of NLCs. Few of these methods listed below(17–20)

1. Melt emulsification ultrasonication
2. Solvent emulsification evaporation
3. High pressure homogenization
4. Microemulsion method
5. Supercritical fluid method
6. Phase inversion method
7. Membrane contractor technique.

Quality by Design (QbD)(21,22)**:**

Quality by Design (QbD) an organised method of developing and producing formulation based on an emphasis on predetermined goals and a thorough comprehension of process and product variability. When applied to nanostructured lipid carriers (NLCs), QbD principles can help ensure the production of high-quality pharmaceutical products with desirable attributes. Here's how QbD can be implemented in the context of nanostructured lipid carriers:

Critical Quality Attributes (CQAs): Identifying and defining the key attributes of the nanostructured lipid carriers that are critical to their quality. A number of variables, including stability, drug loading, release profile, polydispersity index, and particle size, may be included

Critical Material Attributes (CMAs): Identifying and understanding the critical material attributes of the components used in the formulation, such as lipids, surfactants, and drugs. The quality and properties of these materials can significantly impact the performance of NLCs.

Critical Process Parameters (CPPs): Identifying and controlling critical process parameters during the manufacturing process. This includes parameters related to the homogenization process, temperature, pressure, and mixing speed, which can influence the characteristics of NLCs.

Design of Experiments (DOE): The process of designing experiments to systematically investigate how different factors (material and process parameters) affect the critical N LC quality attributes. This helps in optimizing the formulation and manufacturing process.

Risk Assessment: Identifying potential risks associated with the formulation and manufacturing of NLCs. This includes identifying potential failure modes and developing strategies to mitigate or control these risks.

Establish Control Strategies: Establishing control strategies involves developing measures based on a thorough comprehension of important process variables and important material characteristics. These strategies should be designed to ensure that the product consistently meets the predefined quality attributes.

Continuous Monitoring and Improvement: Implement a system for continuous monitoring of the NLC manufacturing process and product quality. This includes in-process monitoring, testing, and analysis to ensure that the product remains within the defined quality parameters.

Documentation and Reporting: Maintain thorough documentation of the entire development process, including rationale for critical decisions, experimental data, and results. This documentation is crucial for regulatory compliance and future process improvements.

By applying QbD principles to nanostructured lipid carriers, pharmaceutical companies can enhance the robustness of their processes, improve product quality, and increase the likelihood of regulatory approval. This approach fosters a more thorough understanding of the relationship between formulation and product performance, leading to more efficient and reliable production processes.

A fishbone diagram is a visual aid that helps identify and arrange the causes of a certain problem or effect. It is also known as an Ishikawa or cause-and-effect diagram. It is named for its resemblance to a fish skeleton, with the "bones" representing different categories of factors contributing to the issue. In the context of nanostructured lipid

carriers (NLCs), use of fishbone diagram to explore and categorize potential factors influencing the quality or performance of the NLCs. Here's the fishbone diagram for NLCs:

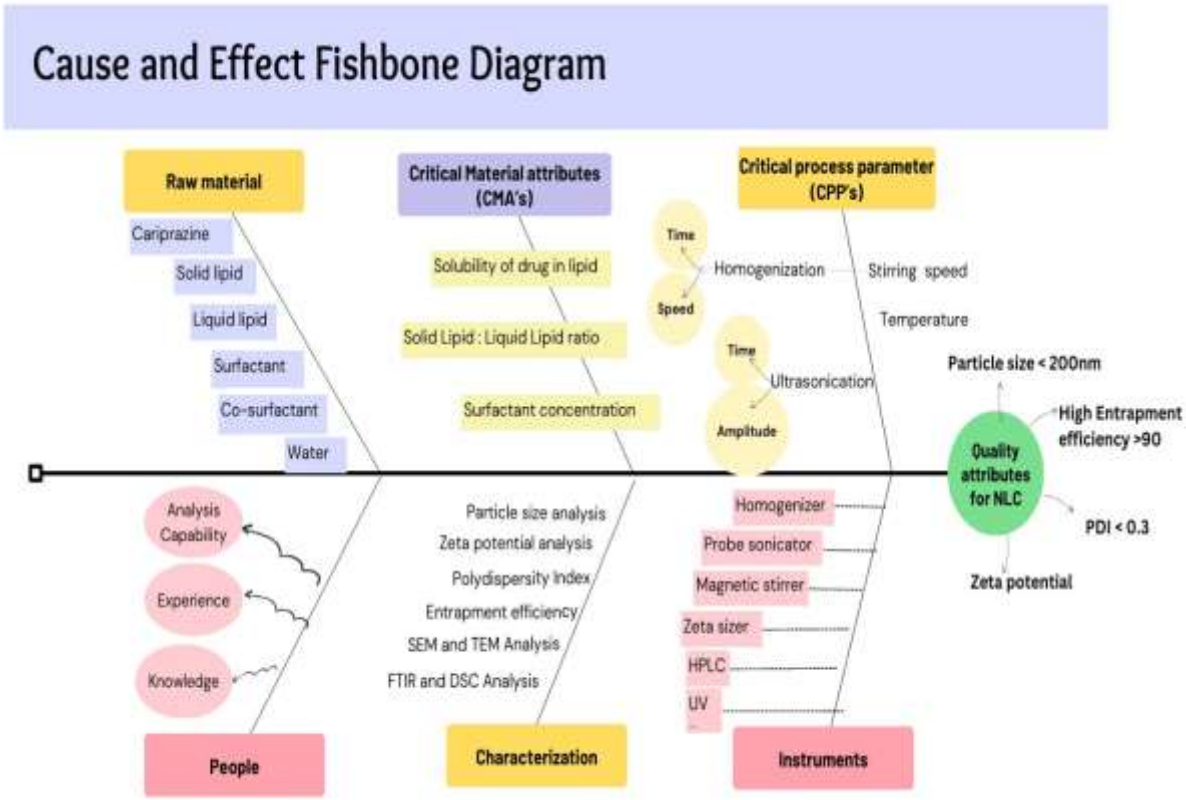


Figure 4: Fishbone Diagram for the development of CPZ-NLC formulation

1.2 REVIEW OF LITERATURE

Maryam Alaayedi (2023): A more effective drug delivery method was developed using an intranasal administration of nanoemulsion since the anticancer drug lomustine had major side effects and poor bioavailability. The most promising drug delivery method, which released 100% of the drug in 15 minutes, has the potential to be a safe and effective replacement for taking the medication orally. Nanoemulsion exhibit acceptable properties as nanocarrier. Drug can bypass the liver metabolism, reduce their toxicity, and potentially target the brain faster(23).

Zhiqiang Tian (2013): Precirol ATO 5, Captex 100, Tween-80, and fenofibrate were the ingredients of NLCs. NLCs were developed using the melting emulsification method**.** Within 15 minutes, almost 90% of the NLCs could be released from the solidified NLC pellets. The method of fluid bed coating utilised for making solidified NLC formulation from liquid formulation (24).

Mingyue Gao (2019): 2,3,5,6- tetramethylpyrazine (TMPP) was model drug for intranasal drug delivery. Tween 80 was used as an absorption enhancer. Tween 80 was a potential excipient to boost drug concentration in both plasma and brain via intranasal route, based on the pharmacokinetic parameter. TMPP can potentially move through the blood-brain barrier passively more effectively if polysorbate 80 (also known as Tween 80) added to formulation which can improve a drug's systemic absorption. This facilitation allows for direct transport from the nasal administration route to the brain (25).

Yasir Mehmood (2023): To enhance the bioavailability and intranasal absorbance of azelastine, a nanosuspension-based nasal spray was prepared. The azelastine nanosuspension demonstrated a rapid drug release pattern within the initial ten minutes.

Within 60 minutes, approximately 97% of the drug had been released, indicating effective and efficient delivery through this formulation. In comparison to a pure reference sample, the drug dissolution and diffusion study showed a 2.0-fold increase(26).

Javed ali et al. (2019): The formulation of an intranasal nanostructured lipid carrier (NLC) for the delivery of lurasidone hydrochloride (LRD) to the brain involved the utilization of the solvent evaporation method. The optimized LRD-NLC exhibited a particle size of 207.4 ± 1.5 nm, a polydispersity index of 0.392 ± 0.15, and an entrapment efficiency of 92.12 ± 1.0%. Transmission and scanning electron microscopy were used to assess the surface morphology and particle size of LRD-NLC. When administered through the nasal route, the formulated NLC exhibited a two-fold enhancement in the amount of LRD present in the brain, surpassing that achieved with the conventional drug solution. The results indicate that utilizing the intranasal route holds promise as an effective technique for releasing the drug to the brain directly. This approach not only improves the drug's effectiveness in treating schizophrenia but also provides a practical alternative to oral medication (27).

Singh et. Al (2016): Asenapine NLC intranasal delivery for the treatment of schizophrenia was found to significantly reduce extrapyramidal side effects while boosting antipsychotic effect(28).

Kokare C et. al (2018): A potential risk associated with second-generation antipsychotic drugs is the possibility of developing agranulocytosis. Kokare et. al formulated olanzapine loaded NLCs via the intranasal route. The NLC was prepared using melt emulsification, and the optimization of the formulation was achieved using the Box-Behnken design methodology. The intranasal formulation of olanzapine NLCs did not exhibit any variation in leukocyte, RBC, or platelet count, based the results of

an in vivo hematological investigation conducted on the drug. So, it can be considered that Olanzapine NLC was safely and effectively delivered for CNS disorders through the nose to the brain (29).

Gowda D V et al (2019): Ziprasidone loaded NLCs was prepared using central composite statistical design. Pharmacokinetic study shows 10-fold increases for NLCs administered via nasal route. Study shows that a potential nose to brain transport of ziprasidone by effective bypassing of the blood brain barrier(30).

Ana Cláudia Paiva-Santos et al (2023): The primary approach to treating psychotic disorders such as bipolar disorder and schizophrenia involves the use of oral antipsychotic medications. Unfortunately, the risk of unfavorable drug reactions poses a challenge to clinical outcomes, leading to patient noncompliance. The blood-brain barrier's restrictive qualities make it difficult to design formulations that aim to improve the delivery of drugs to the brain. The benefits of the intranasal route over oral and intravenous delivery have been proven by recent pharmacokinetic and pharmacodynamic in vivo studies, notwithstanding these difficulties. This route facilitates direct transport of the drug through neuronal pathways from the nasal region to the brain, minimizing systemic side effects and enhancing therapeutic effects.

Various nanosystems, including nanostructured lipid carriers, nanosuspensions polymeric mixed micelles, spanlastics, nanoemulsions, nanoemulgels and niosomes have demonstrated potential for incorporating antipsychotic drugs when administered through the intranasal route. In summary, the intranasal delivery of nanosystems emerges as a highly promising approach for addressing schizophrenia, bipolar disorder, and related conditions (31)

Shreeja Nair et al (2021): It is essential to seek emergency medical attention for acute epileptic seizures as they create a risk of death. Intravenous phenytoin sodium continues

to be the second-line treatment option for the timely management of epileptic seizures. For the treatment of acute seizures, the intranasal delivery of nanolipid carriers (NLCs) containing phenytoin sodium seems to be a successful method. Three different nanosized phenytoin sodium loaded NLCs were developed using the melt emulsification technique, and these were subsequently further characterized. The ex vivo permeation study revealed that nanostructured lipid carriers (NLCs) with a size less than 50 nm exhibited deeper permeation through the olfactory epithelium compared to the control drug solution. In in-vivo pharmacokinetic studies, intranasal administration of phenytoin sodium NLCs with a size <50 nm demonstrated higher drug concentration in the cerebrospinal fluid/brain within 5 minutes, surpassing both the control drug solution and commercially available intravenous phenytoin sodium. The olfactory epithelium plays a crucial role in facilitating direct and rapid drug transport from the nose to the brain (32).

1.3 JUSTIFICATION

Schizophrenia is a chronic mental health condition marked by a variety of cognitive, emotional, and behavioral symptoms. Typically emerging in late adolescence or early adulthood, its precise origin remains incompletely understood. Nonetheless, the consensus suggests that a blend of genetic, biological, environmental, and psychological factors plays a role in its onset. Because atypical antipsychotic drugs cause less extrapyramidal symptoms, tardive dyskinesia, dysphoria, and improve cognition, they are used more often than typical antipsychotic drugs in the therapy for schizophrenia

Cariprazine is a type of atypical antipsychotic medication employed in the management of schizophrenia and bipolar disorder sold under the brand name Vraylar. This drug obtained approval in 2015 from the Food and Drug Administration (FDA) in the United States for treating schizophrenia in adults. Additionally, in 2017, it gained approval for the acute management of adult bipolar disorder-related manic or mixed episodes.

CPZ belongs to BCS (Biopharmaceutics Classification System) Class II, indicating that it has low aqueous solubility. It shows first-pass metabolism. The most often reported side effects included akathisia, EPS, headaches, dizziness, tremors, and gastrointestinal issues. The nanostructured lipid carrier of CPZ is meant to overcome bioavailability and side effects by making at site site-specific. Because of its excellent stability and high drug entrapment efficacy, NLC has been chosen as a drug carrier. NLC's quick absorption by the brain makes it a good option for brain delivery. The biocompatibility of lipid-based carriers results in extremely low toxicity. Improvements in solubility, release modification, nano size, tailored surface, and multifunctionality of nano drug

delivery help to increase bioavailability. Including permeation enhancers into the formulation will facilitate the paracellular transport of the chosen moieties by loosening the barrier's tight joints.

Intranasal drug delivery offers a non-invasive approach to convey medications directly to the brain, bypassing the blood-brain barrier. The administration of nanolipid carrier intranasally bypasses the first-pass metabolism of the drug and directly delivers it to the brain, increasing its bioavailability.

In situations where a patient is unconscious, unable to swallow, or experiences gastrointestinal issues or disorders affecting motility and absorption, the necessity for alternatives to oral delivery becomes evident. Numerous antipsychotics present challenges for oral administration due to factors such as limited bioavailability, hepatic first-pass effects, and gastrointestinal adverse effects. Additionally, the absence of injectable formulations for certain medications is attributed to challenges like inadequate aqueous solubility or unfavorable physical, chemical, or toxicological characteristics. Because of many benefits, including self-administration, safety, and affordability, non-invasive administration is preferred over invasive ones. The nasal route is favored due to its advantageous features, including high blood flow, a porous endothelium membrane, substantial surface area, and the ability to bypass first-pass metabolism.

Thus, in the planned study, efforts will be undertaken to develop and characterize intranasal NLC formulation of Cariprazine using QbD concept.

1.4 OBJECTIVE AND PLANE OF WORK

AIM

To formulate and characterize a nanostructured lipid carrier intranasal spray of Cariprazine hydrochloride.

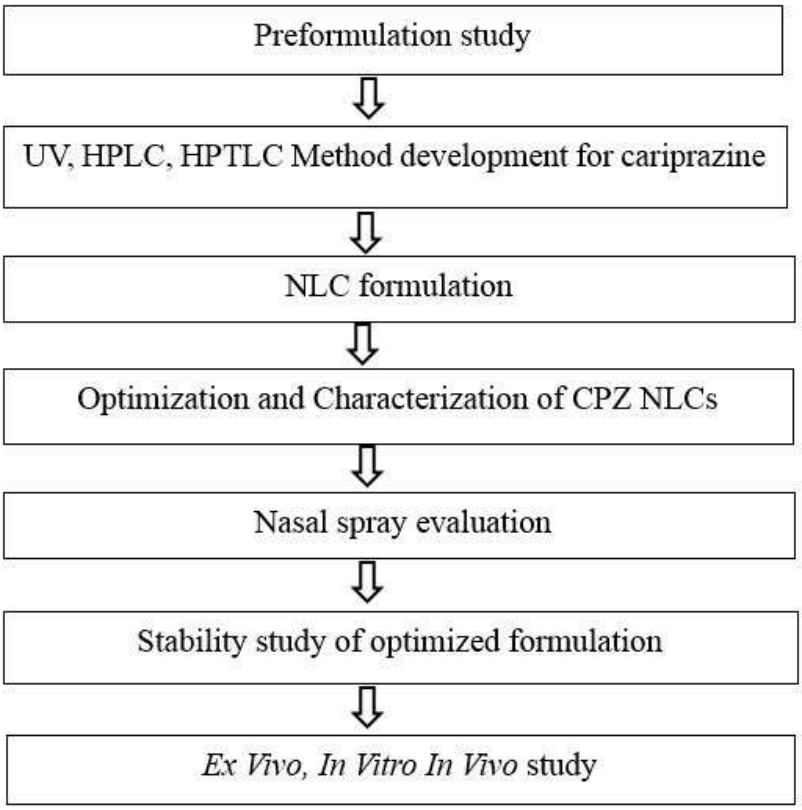
OBJECTIVES

- To formulate and characterize a nanostructured lipid carrier (NLC) spray of Cariprazine for brain targeting via nasal route using QbD approach.
- To access the bioavailability of drug-*In vivo* study

SECONDARY OBJECTIVE

- To develop suitable analytical methods.

PLAN OF WORK



2. MATERIALS AND METHODS

Table 1: List of instruments

S.No	EQUIPMENTS	MANUFACTURER
1.	Electronic Balance	Sartorius
2.	Sonicator	RC system, India.
3.	Milli Q water System	Merck Pvt. Ltd.
4.	Zetasizer Nano ZS	Malvern Instruments Corporation, UK
5.	Magnetic Stirrer	Remi Moors, Mumbai
6.	Brookfield viscometer	Brookfield Engineering
7.	DSC	DSC 60 Shimadzu Corporation, Japan
8.	Digital pH meter	Systronics, India
9.	Probe Sonicator	Fischer Scientific
10	Dialysis membrane	Hi Media Ltd, Mumbai
11.	UV Visible spectrophotometer	UV -1900 Shimadzu Corporation, Japan.
12.	Humidity control chamber	M/s Lab Control equipment, Mumbai
13.	Homogenizer	T25 Ultra Turax, IKA
14.	HPLC	Agilent Technologies
15.	Franz diffusion cell	T. C. Scientific glass work, Harayana
16.	HPTLC	CAMAG, Switzerland
17.	Fourier Transform InfraRed (FTIR)	Bruker Alpha II, USA

Table 2: List of chemicals

S. No	Chemical	Supplier
1	Cariprazine hydrochloride	MSN Labs, Hyderabad
2	Glyceryl monostearate	Mohini Organics PVT. LTD.
3	Capmul MCM	ABITEC, USA
4	Acconon MC8	ABITEC, USA
5	Captex 300	ABITEC, USA
6	Tween 80	Sigma Aldrich, India
7	Compritol 888 ATO	Gattefosse, France
8	Gelot 64	Gattefosse, France
9	Peceol	Gattefosse, France
10	Transcutol P	Gattefosse, France
11	Precirol ATO 5	Gattefosse, France
12	Citric acid	Merck, India
13	Benzalkonium chloride	Merck, India
14	Methanol	Merck, India
15	Trifluoroacetic acid	Fisher Scientific, India
16	Castor oil	Fisher Scientific, India
17	Olive oil	Fisher Scientific, India
18	Coconut oil	Fisher Scientific, India
19	Peppermint oil	Fisher Scientific, India

2.1 Analytical methods for estimation of Cariprazine Hydrochloride

2.1.1 UV spectrophotometric method

Methanol was chosen throughout the study because Cariprazine hydrochloride exhibits solubility in organic solvents, including methanol and dimethyl sulfoxide (DMSO).

A Shimadzu UV 1900 UV spectrophotometer was used to scan a working standard solution containing 10 µg/ml of CPZ between 400 to 200 nm wavelength.

Stock and a working standard solution preparation

10 mg of capricrazine hydrochloride was carefully weighed and added to a 100 ml, dry volumetric flask. Using methanol, the drug was dissolved and diluted to the required amount. This was considered to be a standard stock solution (100 µg/ml). To prepare the working standard, a volume of 1 ml from the stock solution was measured using a pipette and subsequently diluted to a total volume of 10 ml resulting in a concentration of 10 µg/ml. Further required dilution were made to get the desired concentration(33–35).

2.1.2 Development and Validation of RP-HPLC Method for Cariprazine (36–40)

Instrumentation

Agilant 1260 Infinity II, Quaternary pump with degasser (G7111A), auto-injector (G7129A), DAD detector (G7115A), and Openlab Ezchrom software were used.

Chromatographic conditions

The HPLC system was connected to an Agilent Zorbax Bonus – RP column (250 mm × 4.6 mm, 5 µm), facilitated the chromatographic separation. A sample of 10 µL was injected into the system, which operated at a flow rate of 1 ml/min and maintained a

column temperature of 30℃ and detection was at 248 nm. The mobile phase consisted of Methanol and 0.1% Trifluoroacetic Acid in a ratio of 52.5:47.5 v/v.

Preparation of standard stock solution and calibration curve

Cariprazine hydrochloride 5 mg was dissolved into 10 ml of diluent, vortex for 1 min. Sonicate for 5 minutes. Concentration of Cariprazine HCL standard stock solution 500 µg/mL (Diluent -Methanol and 0.1% Trifloroacetic Acid 50:50 v/v) further dilutions are carried out to get the calibration curve ranging from 80 to 120 µg/mL.

The method validation was done on basis of recommendations provided by ICH guidelines(41).

2.1.3. HP-TLC method development and validation for Cariprazine Hydrochloride (42–48)**.**

Instrumentation and chromatographic conditions

This included a visualizer, a twin trough chamber, a nitrogen cylinder connected to a Linomat 5 auto sprayer, and a TLC scanner. The HPTLC system (CAMAG®, Switzerland). Version 3 of the Vision CATS software was used. The stationary phase utilized in the process comprised pre-coated silica gel $60F_{254}$ HPTLC plates, with dimensions of 10×10 cm and 20×10 cm, and a layer thickness of 0.1 mm. These plates were obtained from E Merck KGaA, Darmstadt, Germany. The application of the samples onto the plates was carried out using a 'TLC-Hamilton® glass syringe', sourced from Hamilton-Bonaduz Schweiz, Camag, Switzerland. Samples were applied in the presence of a constant nitrogen gas stream. A twin trough chamber was employed during the development process. (10×10cm and 20×10cm). For the mobile phase, 10 minutes was the ideal chamber saturation time. A deuterium lamp was used

as the radiation source. The wavelength at which the bands were examined was 254 nm.

Method development

The application of samples onto the pre-coated silica gel $60F_{254}$ HPTLC plates was performed using a micro syringe and a Camag Linomat 5 sample applicator with a 6 mm bandwidth. Various concentrations of reference standard solutions for CPZ were prepared, and these solutions were applied as bands on 60 F_{254} plates. A twin trough glass chamber was utilized for plate development. Before the plate was developed in the chamber, the mobile phase was given ten minutes to saturation. The solvent system was allowed to run on around 70 mm of the plate. Once the plate reaches a length of 70 mm, it is allowed to dry with the assistance of an air dryer. Following the drying process, the TLC scanner 4 is employed to analyze the fully dried plate, and the resulting chromatograms are used for result interpretation. The spectra were identified using UV-Range Scanners.

According to ICH Q2 (R1) recommendations, the developed technique was validated. Various parameters were evaluated, encompassing range, linearity, accuracy, precision, specificity, quantification limit, and robustness.

2.2 Preparation of CPZ NLCs by QbD approach

Quality by Design is a systematic concept of product development that focuses on ensuring the quality of the quality of the end product. It involves designing and developing processes with a thorough understanding of how variations in factors can affect the final product's quality (Figure 5). The goal is to build quality into the product from the beginning rather than relying on quality control measures at the end of the manufacturing process.

Table 3: Elements of the QTPP & CQA for development of the CPZ-NLC

QTPP	Target	Justification
Drug delivery system	NLC	Bioavailability enhancement
Dosage form	Nasal spray	Quick onset of action
Route of administration	Nasal	Easy to administer
Drug release	More than 70%	Essential to attain therapeutic activity
CQA		
Particle size	Less than 200nm	Absorption and bioavailability improved
Entrapment Efficiency	More than 90%	Improved therapeutic outcome

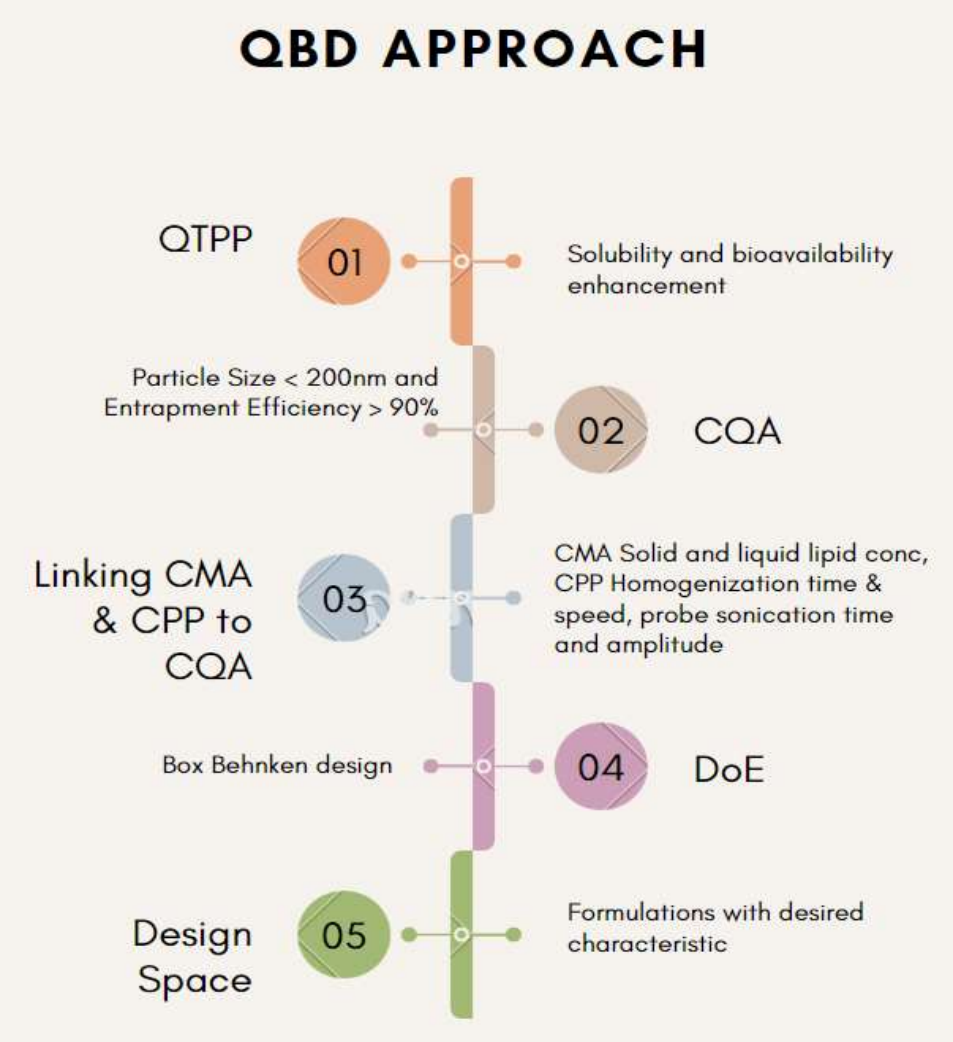


Figure 5: QbD approach

2.2.1 Selection of lipids

In the formulation of Nanostructured Lipid Carriers (NLC), liquid and solid lipids were selected based on their superior solubility for the drug in both components. The drug must have high solubility in lipid and oil to achieve maximum loading. The solubility of CPZ in solid lipids, Comritol 888 ATO, stearic acid, Gelot 64, Glyceryl monostearate and Precirol ATO 5 were determined by melting 1 gram of lipids above their melting point and then integrating small amounts of the drug into the molten lipids. The clarity and transparency of the resulting mixture were assessed, and the point at which transparency was lost indicated the drug's saturation point in the lipid, signifying its solubility limit. (49)

Liquid lipid or oil phase and surfactants screened for this study include Castor oil, Olive oil, Coconut oil, Peppermint oil, Cinnamon oil, Isopropyl Myristate, Acconon MC 8, Captex 300, Capmul MCM, Capryol 90, Kolliphor EL, Labrafac PG, Transcutol P, Transcutol HP, Kolliphor RH 40, Tween 40, Tween 80, Tween 60, Span 80, Span 20. 5 ml of each chosen ingredient were transferred into a glass beaker and positioned on a magnetic stirrer at room temperature to evaluate the solubility of the drug. The drug was added to the oil and surfactant in predetermined amounts, with each addition being stirred for 30 minutes. The point of saturation was identified by the loss of transparency. After that, the resulting mixture underwent centrifugation for a duration of 15 minutes at 15,000 rpm in order to separate the undissolved drug. The drug content was then determined by collecting and analyzing the supernatant by using an HPLC (27,50).

2.2.2 Formulation of CPZ-loaded NLCs

The NLCs were made using the emulsification–ultrasonication technique(28,29,51,52). In brief, predetermined amounts of glyceryl monostearate (solid lipid, SL), Capmul

MCM (liquid lipid, LL), and Cariprazine were blended and heated to 70°C with stirring to form a consistent lipid phase. Following this, Tween 80 and Transcutol P were dissolved in distilled water and heated to 70°C. The heated aqueous phase was then slowly introduced to the lipids, and the resulting mixture was homogenized at 11,000 rpm for 15 minutes using an IKA Ultra-Turrax T18 homogenizer (IKA, Wilmington, NC, USA). To further refine the emulsion, a probe sonicator (Sonics Pvt. Ltd., India) was employed, subjecting the pre-emulsion to ultrasonication at 30% amplitude with a 10:10 on-off cycle for 10 minutes. The finalized formulations were subsequently stored at room temperature.

2.2.3 Optimization of the Nanostructured lipid carrier system using BBD design

Key factors affecting the ideal attributes of the nanostructured lipid carrier intranasal formulation were pinpointed in preliminary screening studies. Initial experiments demonstrated that the favorable attributes of the nanostructured lipid carrier system were directly modulated by the choice of surfactant, liquid lipid, and solid lipid. Constraints were established for each factor that could influence the formulation features, guided by careful observations. Table 4 displays the chosen level of independent variables that were applied to prepare CPZ-NLC.

Table 4: Independent and Dependent Variable Levels in BBD for CPZ-NLC Preparation

Factors/ Independent Variables	**Low (-1)**	**High (+1)**
A=Solid lipid	0.5%	1%
B=Liquid lipid	0.6%	2.5%
C=Surfactant	4%	6%
Dependent variables	Particle size (Y1)	Minimum
	Entrapment efficiency (Y2)	Maximum

The Design Expert 13.0.5.0 software, developed by State-Ease Inc. based in Minneapolis was used to optimize NLC and a three-factor, 3 level Box Behnken design (BBD) design. The design consisted of 15 experimental runs.

2.3 Characterization of CPZ NLC

2.3.1 Particle size, Polydispersity index, and Zeta potential

Employing dynamic light scattering technique (DLS), the Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK) was utilized to assess the zeta potential, polydispersity index (PDI), and particle size of the CPZ-NLCs at 25°C. Subsequently, all samples were diluted with deionized double-distilled water and filtered through a 0.45 μm membrane. The determination was executed in triplicates. If the PDI is less than 0.3, the size distribution is uniform(53,54).

2.3.2 Entrapment efficiency

A predetermined volume of NLC was centrifuged at 15,000 rpm for 30 minutes to assess the amount of CPZ added to the lipid phase and its entrapment efficiency. The resultant supernatant was suitably diluted and tested using UV spectrophotometric analysis at a wavelength of 252 nm (UV 1900, Shimadzu, Japan), and the amount of CPZ entrapped was calculated using the following formula (55,56).

$$\%EE = \frac{Wd - Ws}{Wd} \times 100$$

In the formula, where EE represents entrapment efficiency, Wd denotes the total weight of the drug taken, and Ws signifies drug's weight in the supernatant

2.3.3 Surface Morphology

Transmission Electron Microscopy

The size and morphology studied using a Philips CM 200 (Germany) transmission electron microscope (TEM). The Cariprazine Nanostructured Lipid Carrier (NLC) dispersion was appropriately diluted with distilled water prior to the examination. For imaging purposes, a portion of the diluted dispersion was applied onto a copper grid coated with carbon, and the grid was left to dry. An acceleration voltage of 200 kV was used during the observations (57).

Scanning Electron Microscopy

On a scanning electron microscope (magnification: 50; accelerating voltage: 15.0 kV), images were captured. The analysis was conducted at 25°C. The NLC dispersion was appropriately diluted and ultrasonicated. On the grid, a few drops of the dispersion were placed and allowed to dry. After samples were thoroughly dried, an image was captured.

2.3.4 Differential Scanning Calorimeter Analysis

An aluminium DSC pan was filled with significant quantities (3 mg) of the sample using a DSC loading puncture and sealed. Using a differential scanning calorimeter, the sample was heated at a rate of 5°C per minute and it was scanned between 25° and 300°C in an inert atmosphere of nitrogen. To determine any changes in the drug about enthalpy and interaction with excipient, the DSC was used(28). A differential scanning calorimeter was used to characterize CPZ HCl, GMS CPZ, physical mixture, and CPZ loaded NLCs (DSC-60 Shimadzu).

2.3.5 FT-IR Analysis

The IR spectra of CPZ, physical mixture, CPZ NLC formulation were recorded by Fourier Transform Infrared spectroscopy (Bruker, Model: Alpha II) based on ATR Technique (Attenuation Total Reflection). The sample was kept in an IR cell and runed out 48 scan over sample with IR beam. Sample undergoing molecular vibration on the absorbance of IR beam of light. Vibrational frequencies will be measured in term of wavenumbers (cm^{-1}) versus percentage transmittance. Wavenumbers in the term cm-1 is an indication of a functional group at different positions.

2.3. *In vitro* release study

A dialysis bag containing NLC (2 ml) equivalent to 2 mg was sealed on both ends. The dialysis bag was submerged in a 100 ml phosphate buffer, serving as the dissolution medium. Subsequently, it was stirred at 50 rpm using a magnetic stirrer, maintaining a temperature of 37°C. One milliliter of aliquots were taken out and substituted with a fresh one dissolution medium at predetermined intervals. Samples were withdrawn and,

following appropriate dilutions, spectrophotometric analysis was done at 252 nm. (27,58,59).

2.3.7 Study of *ex vivo* permeation using sheep nasal mucosa

The nasal mucosa was acquired from a freshly sacrificed sheep at a nearby slaughterhouse. The excised tissue was then placed in ice-cold PBS (pH 6.4) and maintained in an aerated condition. The excised nasal mucosa was cut into thin, 0.2 mm-thick pieces to place the samples on the donor and receptor parts of the diffusion cell. The mounted mucosa sample was stabilized for 15 minutes at 37± 1°C using PBS (pH 6.4) treatment. A 1 mL sample of Nanostructured Lipid Carriers (NLCs) was introduced into the donor compartment, while the receptor compartment was filled with phosphate buffer (pH 6.4). 1 mL aliquots were removed from the receiver compartment at predetermined intervals and replaced with PBS (pH 6.4) buffer. Withdrawn samples were diluted and tested using UV spectrophotometric analysis (UV1900, Shimadzu, Japan) at a wavelength of 252 nm. Triplicates of each measurement are made, and the data is displayed as mean±SD (60,61).

2.3.8 Studies on Nasal Ciliotoxicity

Freshly isolated sheep mucosa was used for the nasal ciliotoxicity investigations. Each tissue sample underwent a two-hour exposure to optimized CPZ NLCs, PBS pH 6.4 (utilized as the negative control), and isopropyl alcohol (employed as the positive control). The resulting tissue samples were then preserved in 10% formalin solution and stained with hematoxylin and eosin for histological imaging (60,62,63).

2.3.9 Drug content

The 1ml of NLCs loaded with cariprazine was transferred into 10 ml volumetric flasks, dissolved, and then further diluted to reach the required volume with methanol. The

solution was immersed in an ultrasonic bath for 15 minutes to achieve complete drug dissolution. Following this, the samples were filtered through a 45 µm membrane and the sample was analysed using the developed HP-TLC method(47,64).

2.3.10 In vivo Pharmacokinetic study

With permission from the institutional ethical committee of KLE College of Pharmacy, Belagavi, healthy male albino Wister rats weighing 200–250 g were utilized for pharmacokinetic research (221/Po/Re/S/2000/CPCSEA RNo 34). The experimental animals were housed at a temperature of 25°C in standard laboratory conditions. Animals are divided in 3 groups having each 6 animals. Group 1- Intranasal CPZ NLC, Group 2- CPZ suspension IN, Group 3- CPZ Oral. The dose was 1 mg/kg. Five animals each time point were sacrificed in each group to collect their blood and brain. An anticoagulant-containing Eppendorf tube was used to collect blood samples. Blood samples were centrifuged for 15 minutes at 4°C at 15,000 rpm to separate the plasma. Brain samples were collected, and using a tissue homogenizer, they were homogenized in phosphate buffer. Brain homogenate and plasma samples were extracted by acetonitrile. The cariprazine quantification in plasma and brain samples was done by HPLC system(27,28,65).

2.3.11 Stability study

Short term stability study was carried out to check physical stability of formulation. CPZ-NLCs (F6) was kept under accelerated conditions and room temperature for three months. At designated time interval sample was analyzed for particle size, poly dispersity index, and entrapment efficiency.

2.4 Nasal spray evaluation(66–68)

A digital pH meter was used to measure the pH of formulations.

Using a Brookfield viscometer, the viscosity of formulations was determined.

Clarity test - The dispersion in the test tube was examined under light against a black and white background using a clarity testing device.

Pump delivery - The formulation was put into the bottle (weight of bottle noted down) and actuated ten times in a reweighed bottle. The weight of the bottle was measured once more after ten actuations, and the difference was computed.

A study of content uniformity is utilized to determine the drug amount in various formulations. 1 ml of spray solution was accurately measured and transferred to a 10 ml volumetric flask, and 5-7 ml of diluent was mixed for 2 minutes and make up the volume to the mark with diluent. Analyzed with HPLC system.

RESULT & DISCUSSION

3.1 Analytical methods for estimation of Cariprazine Hydrochloride

3.1.1 UV Method Development for Cariprazine Hydrochloride

It had been found that Cariprazine hydrochloride was stable and soluble in methanol at room temperature. As a result, this solvent was utilized to determine the appropriate detection wavelength and working concentration of the standard.

The sample was scanned by using methanol as a blank between the wavelength ranges 400-200nm. The UV spectra were displayed in Figure 6 and the absorption curve revealed two distinct absorption maxima at 217 nm and 252 nm. Dilution of the working sample solution with methanol to give concentration range 10-50 µg/ml. The UV spectra of CPZ were obtained by scanning between 400 -200 nm. The wavelength was selected 252 nm.

There was no interference shown by the solvent (methanol) spectrum at 252 nm. The UV spectrum of Cariprazine Hydrochloride is represented in Figure 6.

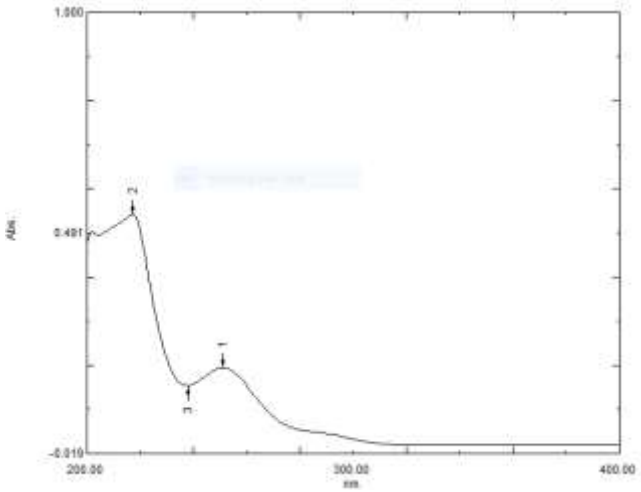


Figure 6: Cariprazine hydrochloride standard UV spectrum

Figure 7 displays the overlay spectrum of Cariprazine hydrochloride's linearity, whereas Figure 8 displays the standard calibration curve.

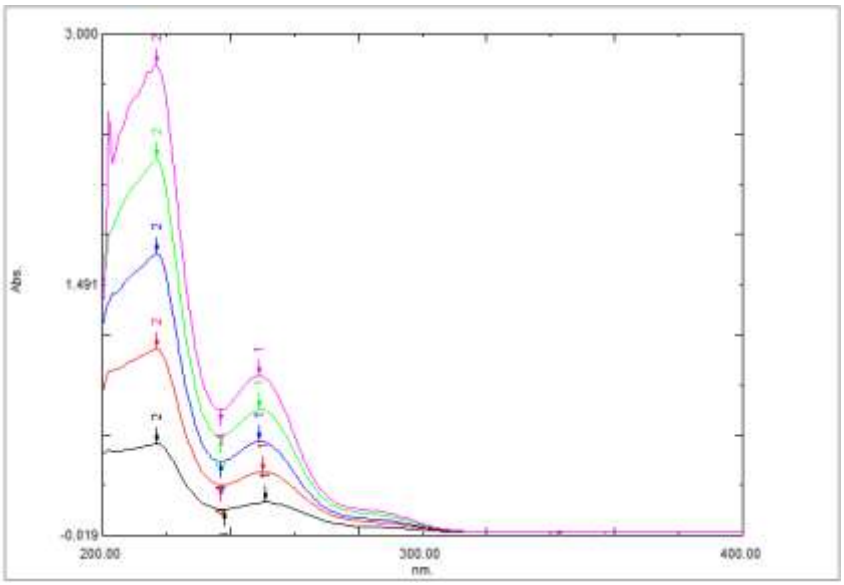


Figure 7: Overlay spectrum of cariprazine hydrochloride

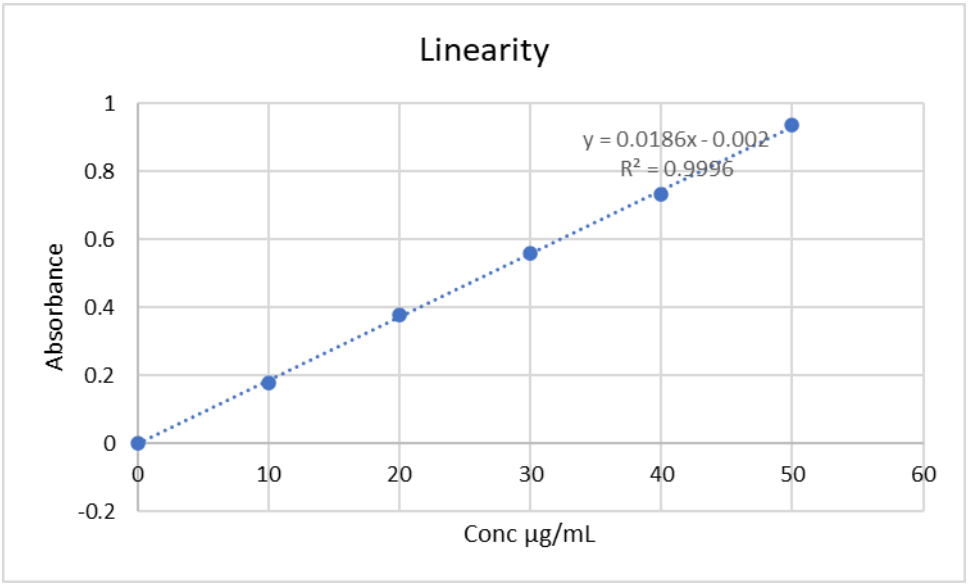


Figure 8: Cariprazine hydrochloride linearity graph

3.1.2 HPLC method development and validation of CPZ

HPLC analysis was performed for CPZ and retention time was found to be 3.85 at 248 nm. The HPLC chromatogram for CPZ were shown in Figure 9. The system suitability were found to be within acceptable limits. The number of theoretical plates (N > 2000), the tailing factor (T < 2%), and the relative standard deviation of the peak area (% RSD < 2%). The regression analysis data for the calibration curve shows a linear relation over the concentration range of 80-120 µg/mL for CPZ. The correlation coefficient was

found to be R^2=0.999. The regression equation for the calibration curve was y= 17117x – 19735 as shown in Figure 10. The % RSD value for the precision of the method (repeatability) was 0.14%. The LOD and LOQ values were 44.8 ng/mL and 135.6 ng/mL respectively. Minor variations in flow rate, mobile phase composition, and column temperature exhibit negligible impact on chromatographic parameters. The chromatogram displaying the linearity overlay of Cariprazine hydrochloride is presented in Figure 11.

Table 5: Final Chromatographic Conditions

Sr. No	Parameter	Condition
1	HPLC Instrument	Agilent 1260 Infinity II
2	Column	Agilent Zorbax Bonus RP (250 x 4.6 mm, 5 micron)
3	Wavelength	248 nm
4	Mobile Phase	52.5 Methanol: 47.5 0.1% Trifluoroacetic acid in water
5	Diluent	Methanol: 0.1% Trifluoroacetic acid in water (50:50)
6	Run time	8 minutes
7	Injection Volume	10 µl
8	Column Oven temperature	30˚C
9	Flow Rate	1 ml/min

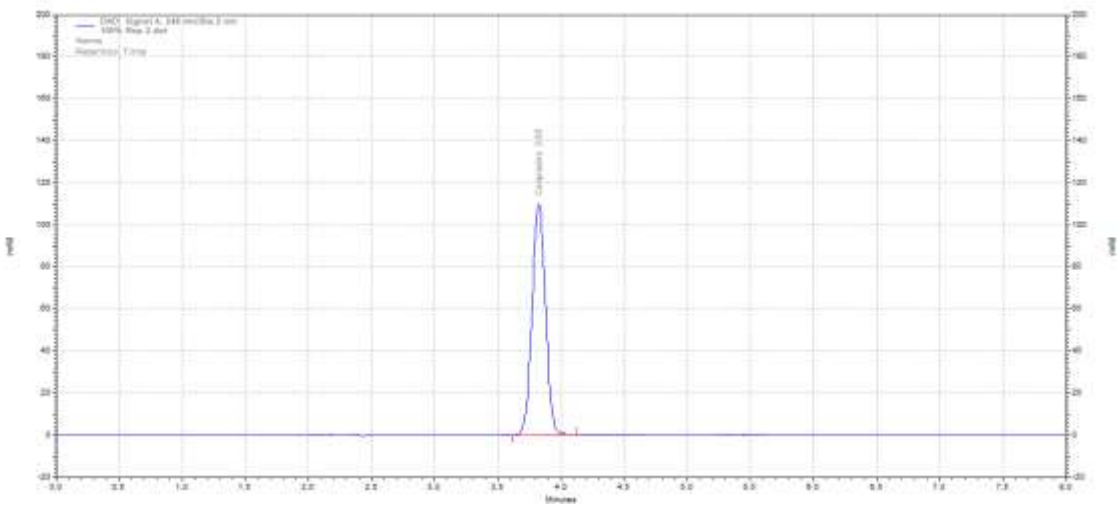


Figure 9: HPLC chromatogram for CPZ

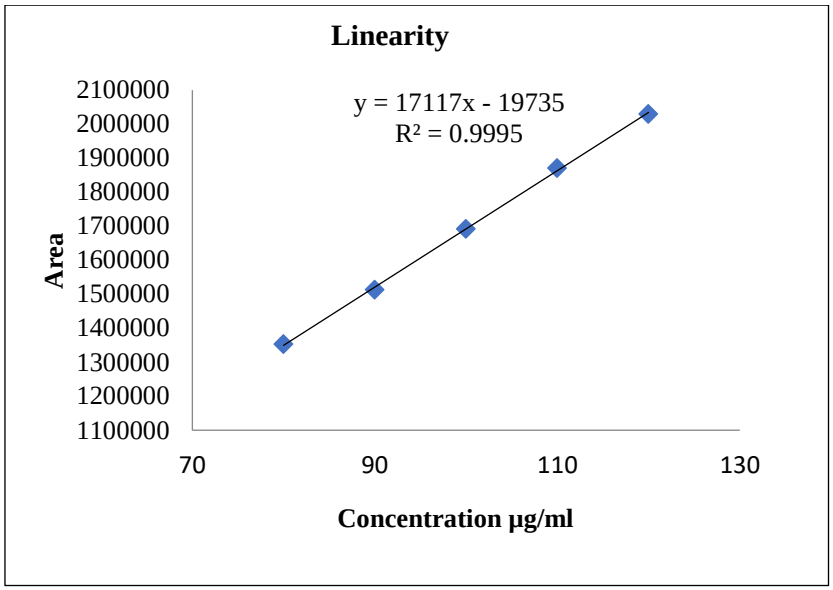


Figure 10: Calibration curve of CPZ by HPLC

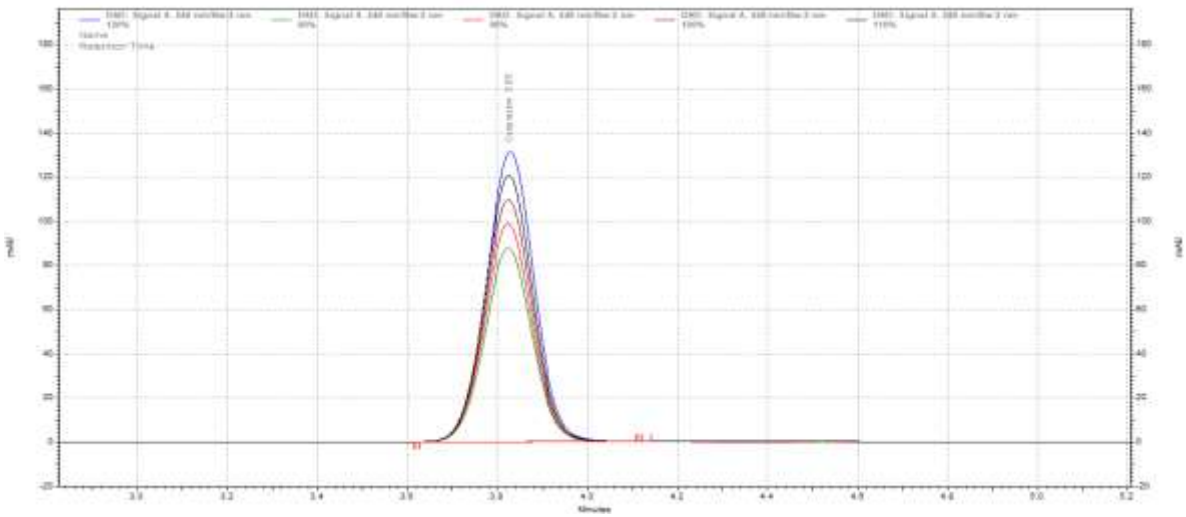


Figure 11: Linearity overlay chromatogram of cariprazine hydrochloride

3.1.3 HP-TLC method development for CPZ

HP-TLC (High-Performance Thin Layer Chromatography) analysis was performed for CPZ. The wavelength at which the highest absorbance of Cariprazine hydrochloride was observed, 253 nm, was chosen for analysis (Figure 13). The mobile phase consisted of Toluene: Methanol (7:3 v/v). A 10-minute chamber saturation time has been given. Retention time was found to be 0.64. The chromatogram of both CPZ and CPZ NLCs

were shown in Figures 14,15 resp. The ICH Q2 (R1) guideline was followed in the validation of the method.

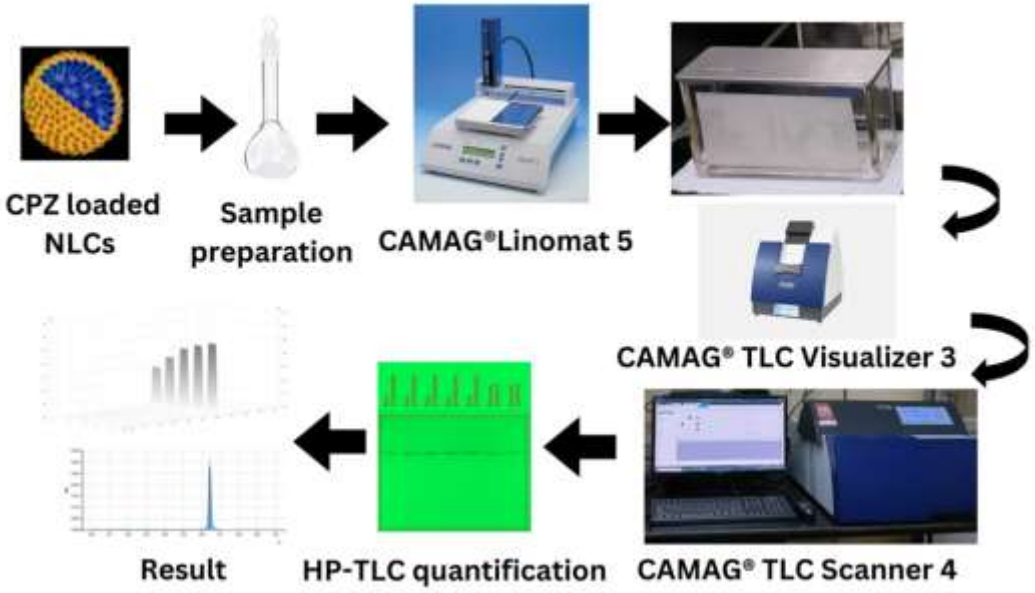


Figure 12: A schematic diagram for the HP-TLC instrument along with method development for determination of CPZ

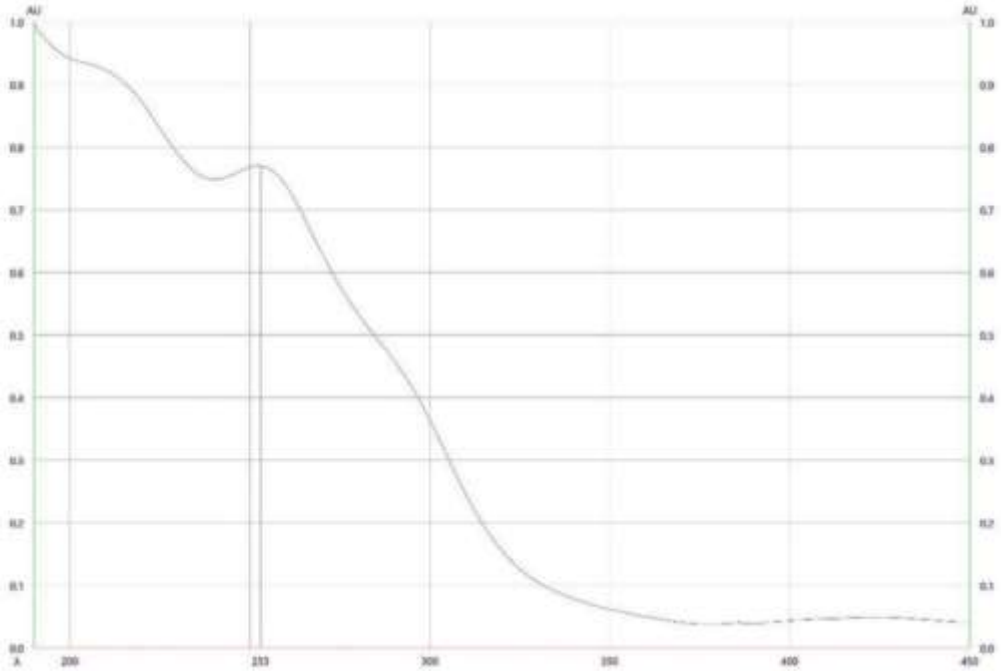


Figure 13: CPZ Spectra

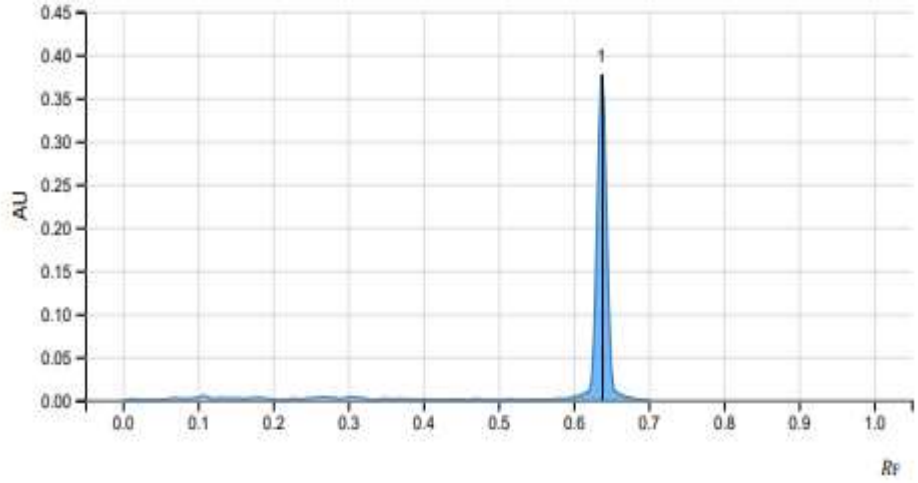


Figure 14: CPZ Typical chromatogram

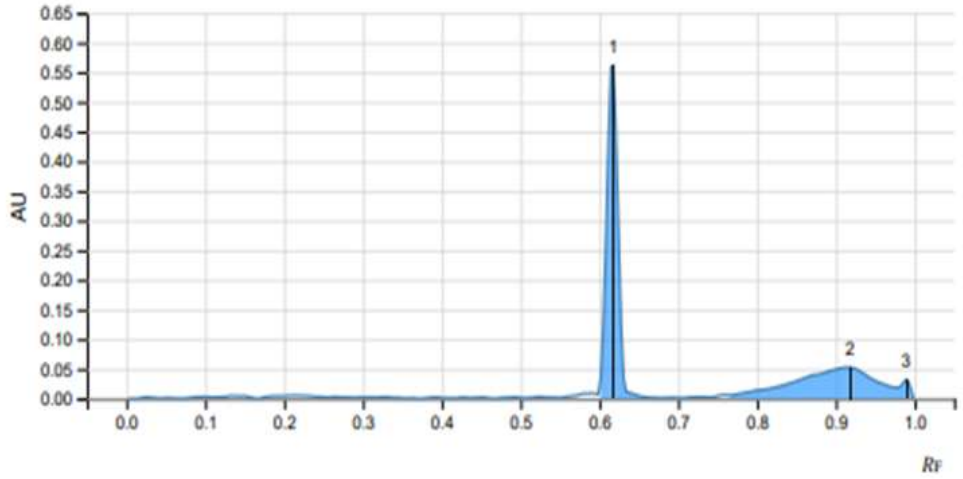


Figure 15: CPZ NLC formulation at 253 nm

Within the concentration range of 1–5 µL/band for CPZ, a remarkable linear correlation is observed with a regression coefficient of 0.993. Figure 16 provides a visual representation of the linearity on the HP-TLC developed plate. The drug exhibits a well-defined band at Rf 0.64, and the corresponding linear regression equation is expressed as Y = 0.0008X + 0.0042 (Figure 17). The linearity chromatogram is displayed in three dimensions in Figure 18 using the Vision CATS software. It was found that the limit of detection and quantification were 0.50 µg/band and 1.52 µg/band, respectively. The % RSD for intraday and interday precision (n=6) was 0.27 and 0.24, which is less than 2%. Variations in the mobile phase volume and saturation

time resulted in a percent relative standard deviation (RSD) within 2%, indicating the robustness of the new technique.

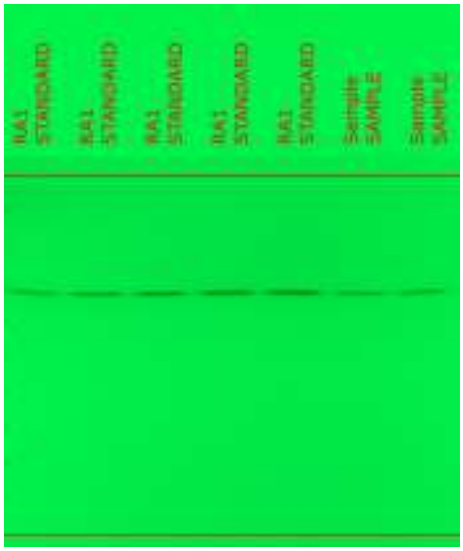


Figure 16: Developed plate of linearity

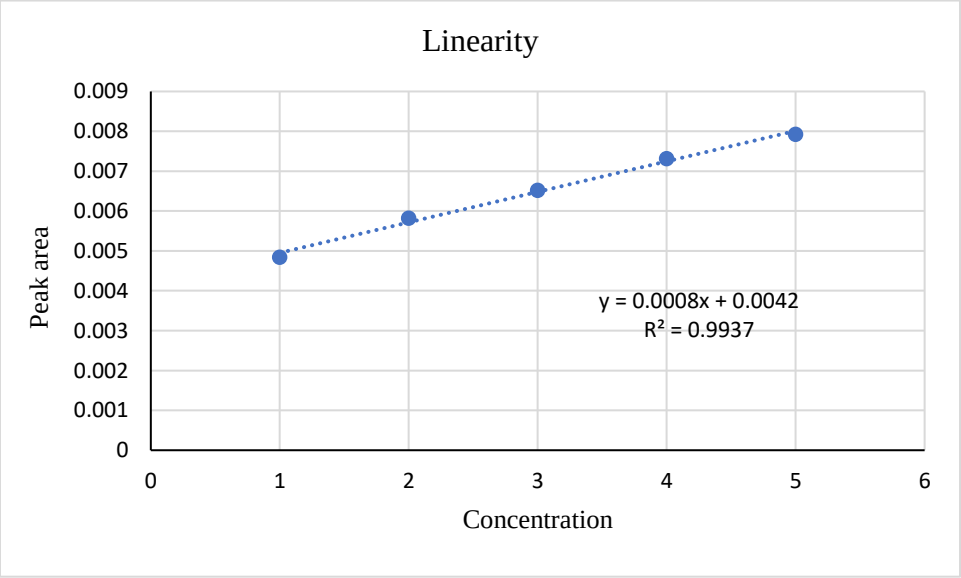


Figure 17: Linear regression of CPZ standards

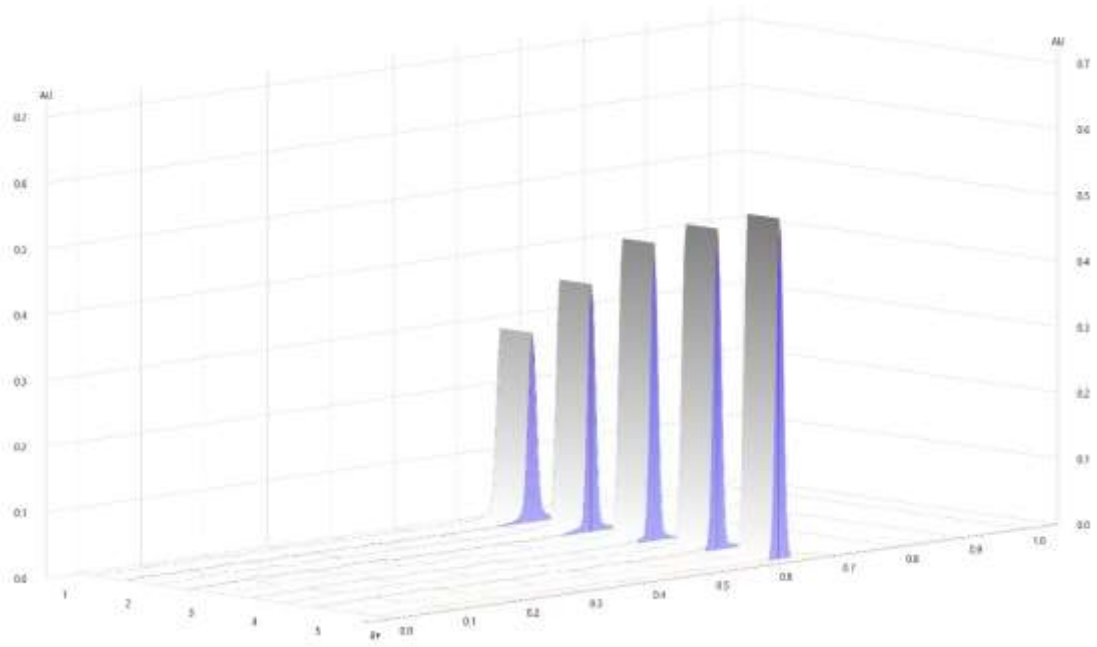


Figure 18: Linearity chromatograms of CPZ (1, 2, 3, 4,5 µL/band TRACK 1-5) in 3D view

3.2 Preparation of CPZ NLCs by QbD approach

3.2.1 Lipid selection

Glyceryl monostearate (GMS) and Capmul MCM were selected as the solid and liquid lipids, respectively, considering the drug's maximum solubility in each. The solubility of the drug in different lipids is illustrated in Figure 19 and Figure 20.

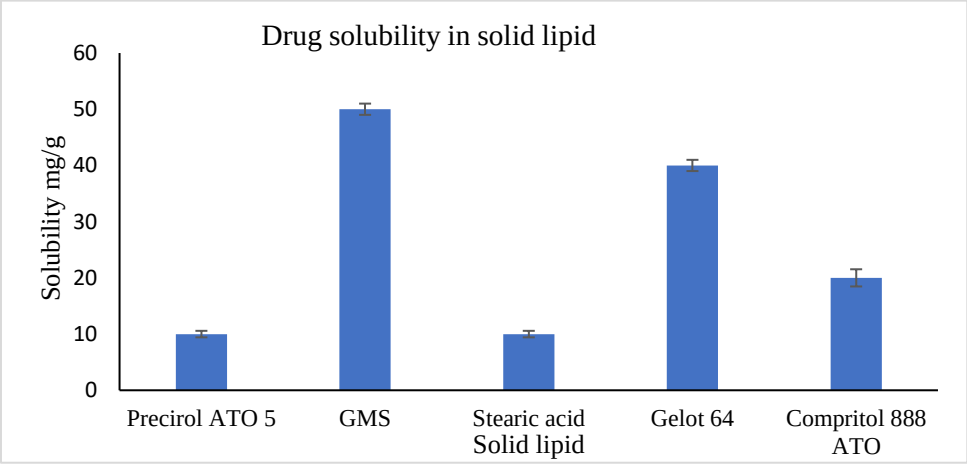


Figure 19: Drug solubility in various solid lipids

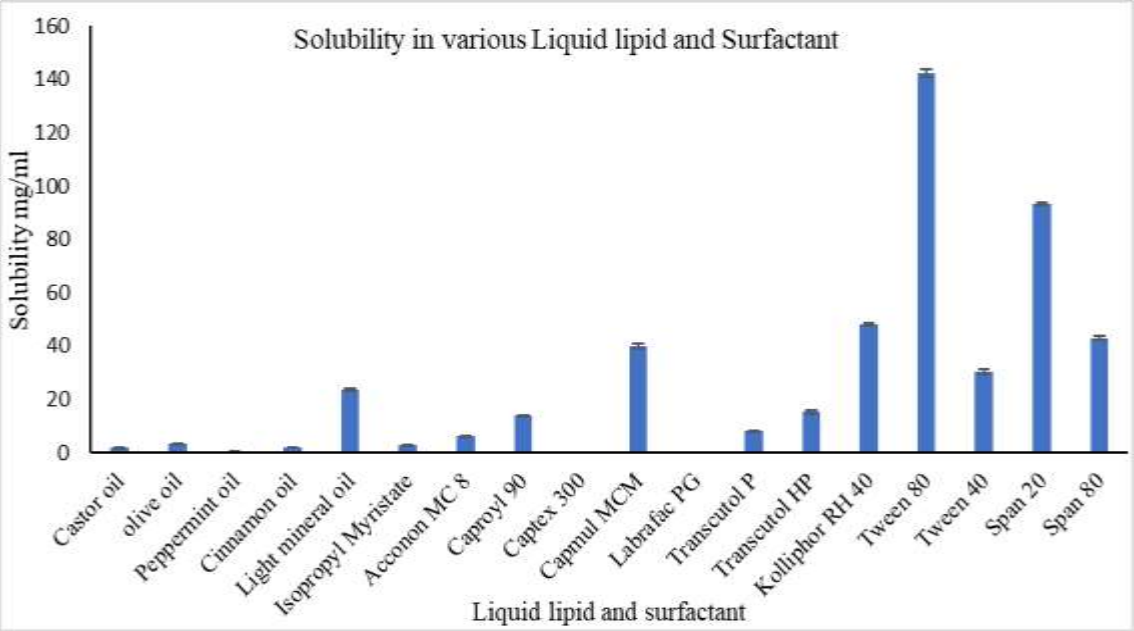


Figure 20: Drug solubility in different liquid lipid and surfactant

3.2.2 Formulation of Nanostructured Lipid Carriers (NLCs) Loaded with CPZ

The melt emulsification method was employed to formulate nanostructured lipid carriers for CPZ. This choice was influenced by the recognition that the concentrations of solid lipid, liquid lipid, and surfactant are pivotal factors impacting both particle size and entrapment efficiency. Box Behnken design was employed to optimize the formulation. The flowchart of the preparation of CPZ NLCs is shown in Figure 21.

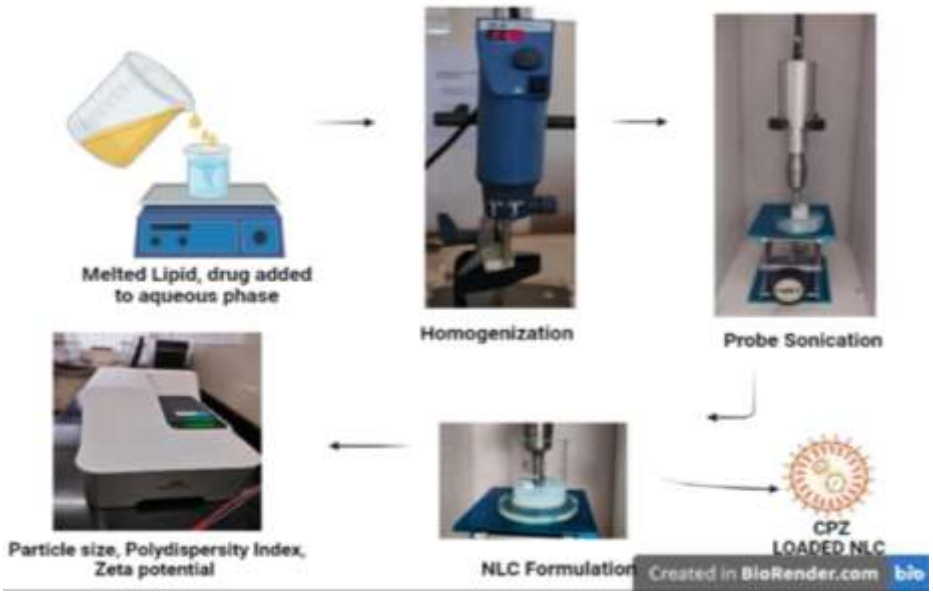


Figure 21: The flowchart of the preparation process of CPZ-NLC

3.2.3 Optimization of the Nanostructured Lipid Carrier System using BBD Design

15 experimental runs with three center points have been generated for formulations using BBD design. Table 6 displays the responses from these runs. The two response variables, Y1 (particle size) measured in nanometers and Y2 (entrapment efficiency) in percentage, exhibit ranges of 173–252.5 nm and 81.45–98.32%, respectively. Table 7 provides information on the percentage coefficient of variation, standard deviation (SD), and R^2 for each response. A three-dimensional graph, Figure 22 and Figure 23 displays the effect of the independent variable's solid lipid, liquid lipid, and surfactant concentration.

Table 6: Observed responses in BBD design

Batch	Solid lipid GMS (%)	Liquid lipid Capmul MCM (%)	Surfactant Tween 80 (%)	Particle size (nm)	Entrapment efficiency (%)
F1	0.75	2.5	4	252.2±1.3	98.02±0.6
F2	1.00	1.55	6	196.1±1.18	98.12±1.4
F3	0.5	1.55	6	180.4±1.2	90.14±0.57
F4	0.5	1.55	4	245.3±0.5	92.43±1
F5	0.75	0.6	6	178.6±0.7	84.04±1.5
F6	0.75	1.55	5	173.3±0.6	96.1±0.57
F7	0.75	2.5	6	206±1.0	97.12±1.5
F8	1.00	2.5	5	198.4±1.7	98.32±2.08
F9	1.00	0.6	5	197±0.5	84.1±1.0
F10	0.75	0.6	4	246.1±1.15	81.45±0.63
F11	0.75	1.55	5	178±0.5	97±0.57
F12	0.5	2.5	5	194±1.2	95.09±1.0
F13	0.75	1.55	5	173.9±0.3	97.14±0.50
F14	1.00	1.55	4	248±1.15	92.78±0.65
F15	0.5	0.6	5	197±1.1	84±0.52

Influence of Independent Variables on Particle Size: Y1

Response 1 (Y1): The particle size observed in all 15 experimental runs ranged from 173.3 to 252.2 nm.

Particle Size =+174.97+2.85A+3.99B-

28.81C+1.10AB+3.25AC+5.33BC+9.18A^2+12.45B^2+33.3C^2……. Equation 1

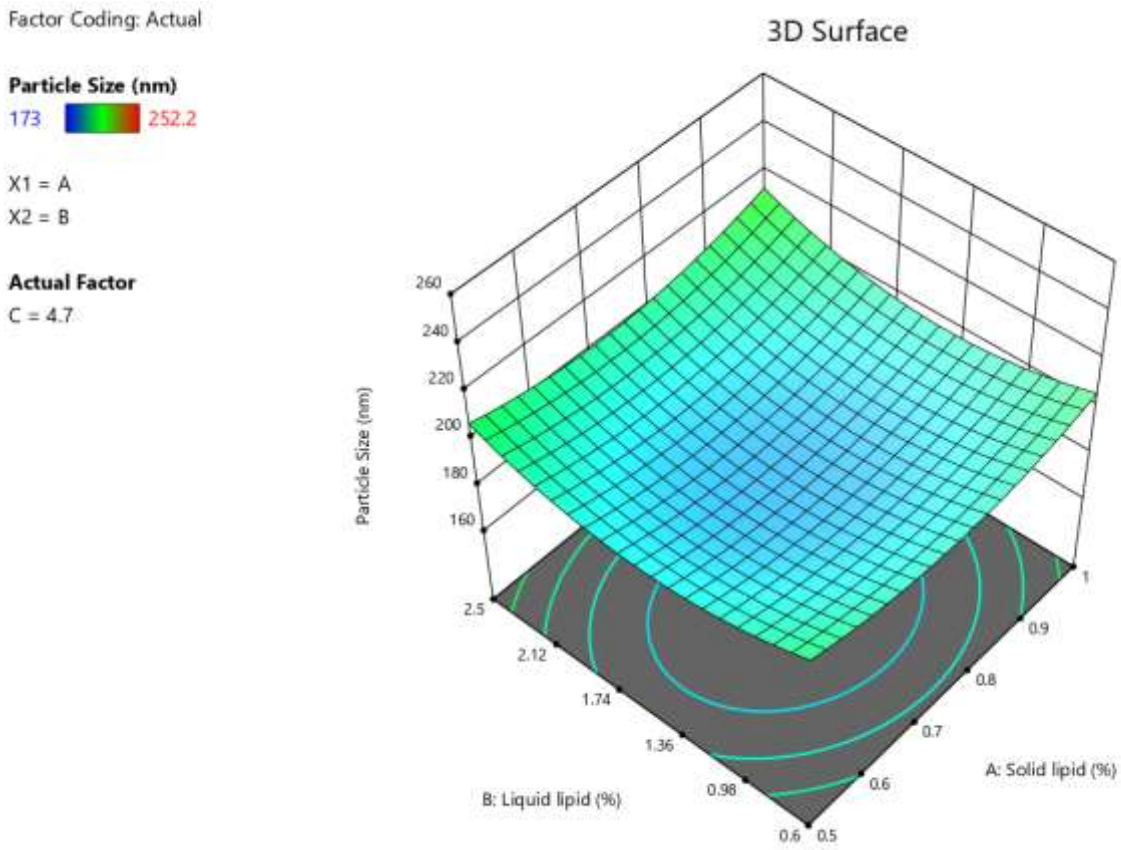


Figure 22: Three-dimensional response surface plot illustrating the impact of independent variables on particle size.

Based on the equation above, it was determined that the particle size of NLC grows along with changes in the concentrations of liquid and solid lipids. Particle size was, however, decreased as surfactant concentration increased.

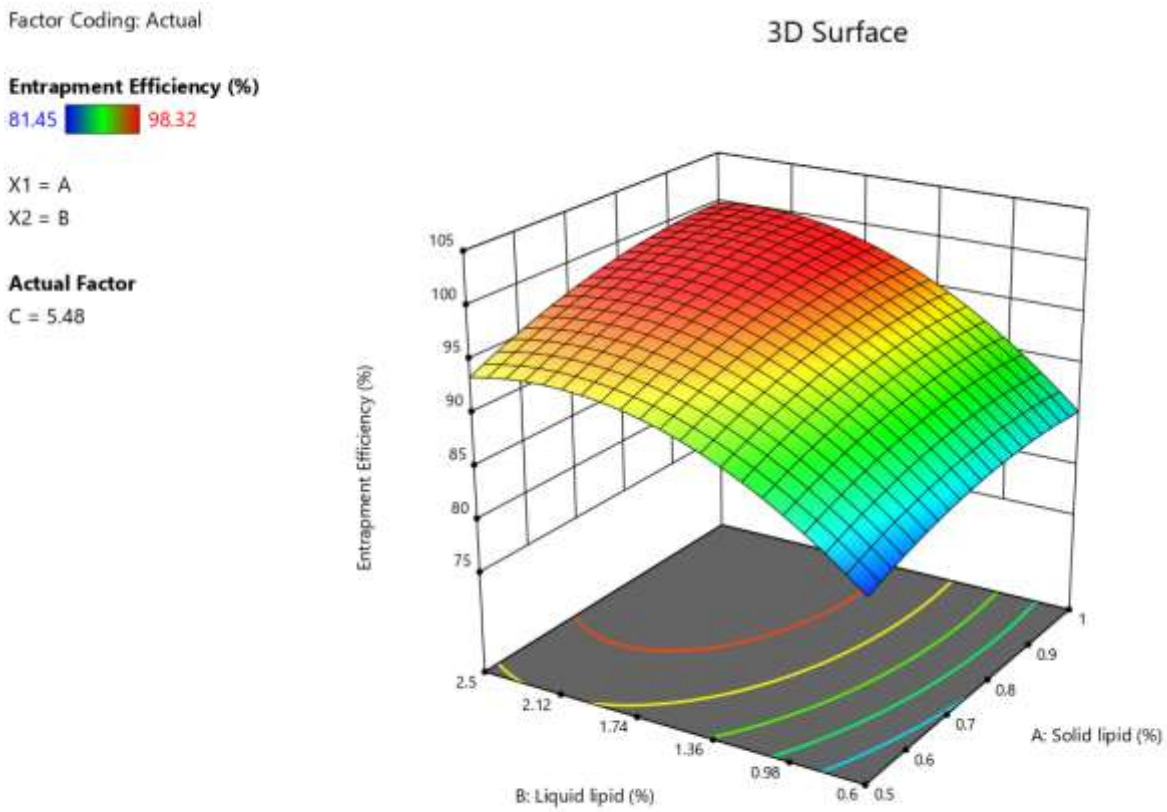


Figure 23: Three-dimensional response surface plot illustrating the impact of independent variables on entrapment efficiency

Influence of Independent Variables on Entrapment Efficiency: Y2

The entrapment efficiency for all 15 formulations fell within the range of 84-98.32%.

EE = +96.71+1.46A+6.87B+0.5925C+0.7825AB+1.91AC-0.8725BC1.56A^2-

4.77$B^2$1.78C^2 ……. Equation 2

From equation 2, the results of the research indicate that entrapment efficiency increases with lipid concentration. The entrapment efficiency is influenced by the amount of liquid lipid present, since a higher lipid content results in a higher entrapment efficiency.

Table 7: Summary of Regression Analysis

Models	SD	R^2	Adjusted R^2	Predicted R^2	Press	Remark
Response 1: Particle Size						
Linear	21.20	0.5802	0.4657	0.3195	8014.20	
2FI	24.45	0.5939	0.2893	0.2443	14654.32	
Quadratic	6.23	0.9835	0.9539	0.7531	2907.20	Suggested
Response 2: Entrapment Efficiency						
Linear	3.34	0.7643	0.7000	0.6041	205.82	
2FI	3.58	0.8028	0.6550	0.4081	307.74	
Quadratic	1.14	0.9875	0.9651	0.8209	93.11	Suggested

SD- Standard deviation, Press-Predicted residual error sum of square, 2FI- 2 Factor interaction, R^2-Multiple correlation coefficients

The optimized batch with CPZ NLC (F6) had a mean particle size of 173.3± 0.6 nm. The optimized NLC formulation had a 96.1± 0.57 % entrapment efficiency. Particle size (174.2 nm) and entrapment efficiency (96.53%), these values closely aligned with the predicted values derived from the BBD design. To characterize the morphology, the optimized formulation underwent additional scanning electron microscopy (SEM) and transmission electron microscopy (TEM) analyses. The details of the optimized NLC preparation formula are presented in Table 8. Transcutol P has been used as Co-surfactant as well as a penetration enhancer, sodium hydroxide and citric acid used to adjust the pH of formulation. Benzalkonium Chloride used as preservative.

Table 8: Optimized Formula

Material	Amount in %
Cariprazine HCL	0.1
Solid lipid GMS	0.75
Liquid lipid Capmul MCM	1.5
Tween 80	5
Transcutol P	1
Sodium Hydroxide (pH)	qs
Citric acid (pH)	qs
Benzalkonium Chloride	0.02

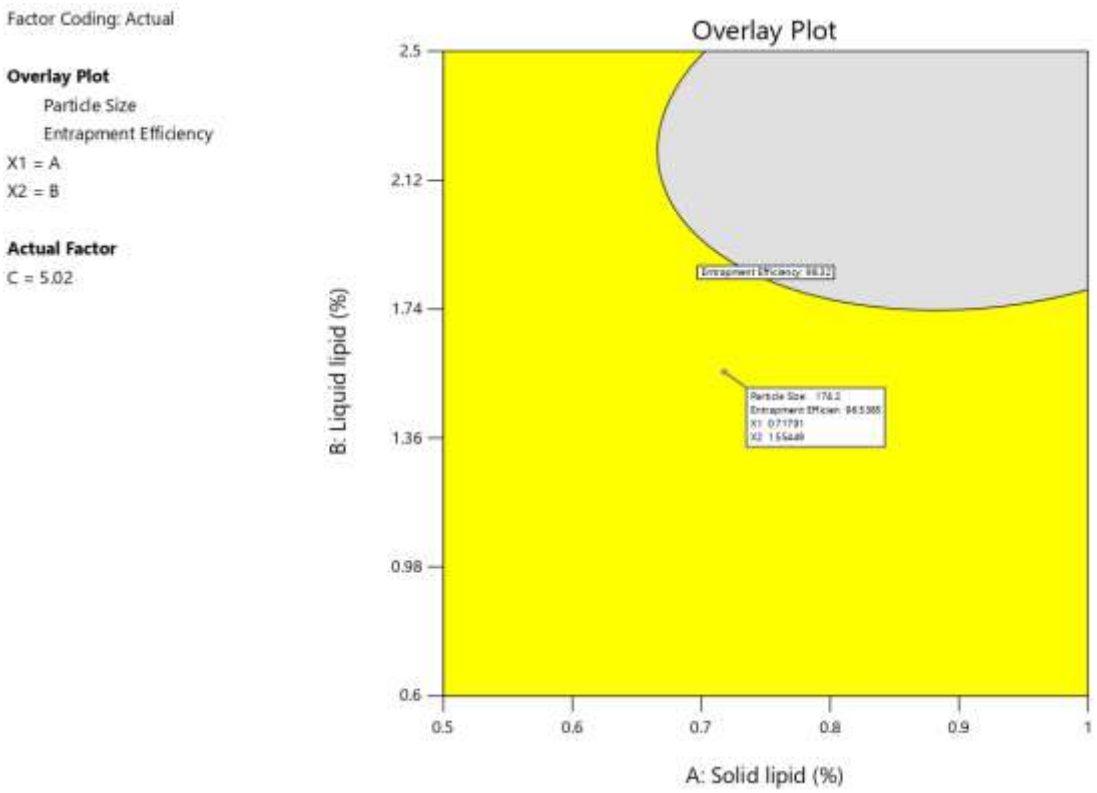


Figure 24: Overlay plot (Design space)

3.3 Characterization of CPZ-loaded Nanostructured Lipid Carriers (NLCs)

3.3.1 Particle size, Polydispersity index (PDI), and Zeta potential (ZP)

The intranasal route of drug administration is influenced by the particle size of the formulation. The absorption through the nasal route is significantly influenced by particle size. As the liquid lipid increased, the particle size decreased. The mean particle size of the optimized formulation was determined to be 173.3± 0.6 nm, with a mean Polydispersity Index (PDI) of 0.30± 0.6, signifying a uniform distribution of the drug. Zeta potential 6.22 ± 2.5 mV. Zeta potential distribution and particle size of the optimized batch (F6) were shown in Figure 25. The delivery of drugs via the intranasal route is influenced by the formulation's particle size. Drug release via monodispersed NLC formulation is indicated by a lower PDI, which suggests a homogeneous distribution of NLC particles. The intranasal route of brain delivery is facilitated by the greater surface area of the nanosized formulation. The surface charge of nanoparticles is analyzed using zeta potential characterization. The hydrophobic tail of Tween 80, a hydrophilic non-ionic surfactant, prevents the particles from aggregating together due to static repulsion, which facilitates steric stabilization of the formulation (29).

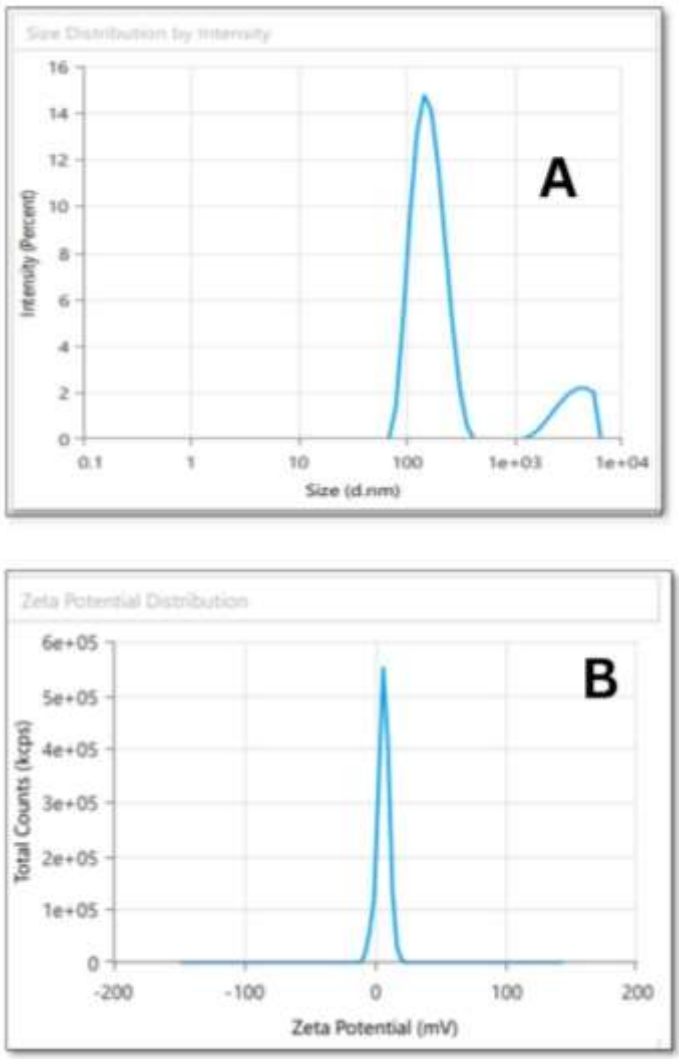


Figure 25: Particle size distribution (A) and ZP (B) of optimized formulation

3.3.2 Entrapment efficiency

The nature of an oil phase determines the entrapment efficiency (%) of a formulation. Because more drug particles were caught in the lipid's core, the maximum amount of total lipid can entrap more drugs and result in a higher percentage of EE. A formula that has been optimized shows 96.1±0.57% EE.

3.3.3 Surface Morphology

Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM)

Utilizing TEM and SEM, an examination of the external morphological structure of the prepared NLC was conducted, revealing the spherical shape of the NLCs. The size of the NLC, determined by SEM and TEM, ranged between 160 and 200 nm, aligning well with the particle size measured by a particle size analyzer using dynamic light scattering.

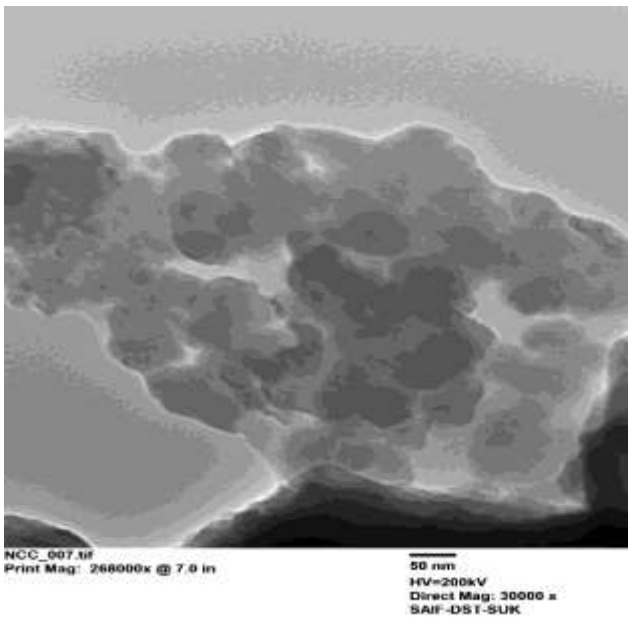


Figure 26: SEM image of optimized CPZ loaded formulation

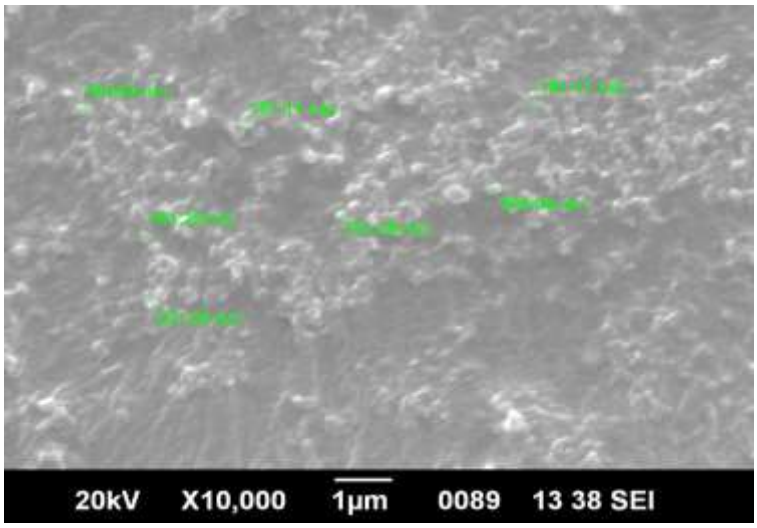


Figure 27: TEM image of optimized CPZ loaded formulation

3.3.4 Analysis by Differential Scanning Calorimetry (DSC)

DSC thermogram of CPZ HCl, GMS CPZ mixture, Physical mixture, and CPZ loaded NLCs are shown in Figure 28. The crystalline nature of CPZ HCl (T onset = 263.20) and GMS (T onset = 63.71) was shown by a strong endothermic peak that corresponds to their melting point. A broad, asymmetric melting peak was seen in the NLCs loaded with CPZ thermogram. The absence of a CPZ melting peak in CPZ NLC could be attributed to CPZ HCL's molecularly dispersed inside the lipid matrix. A broader endothermic peak was associated with the possible effect of liquid lipid and surfactant on the GMS crystal lattice (28).

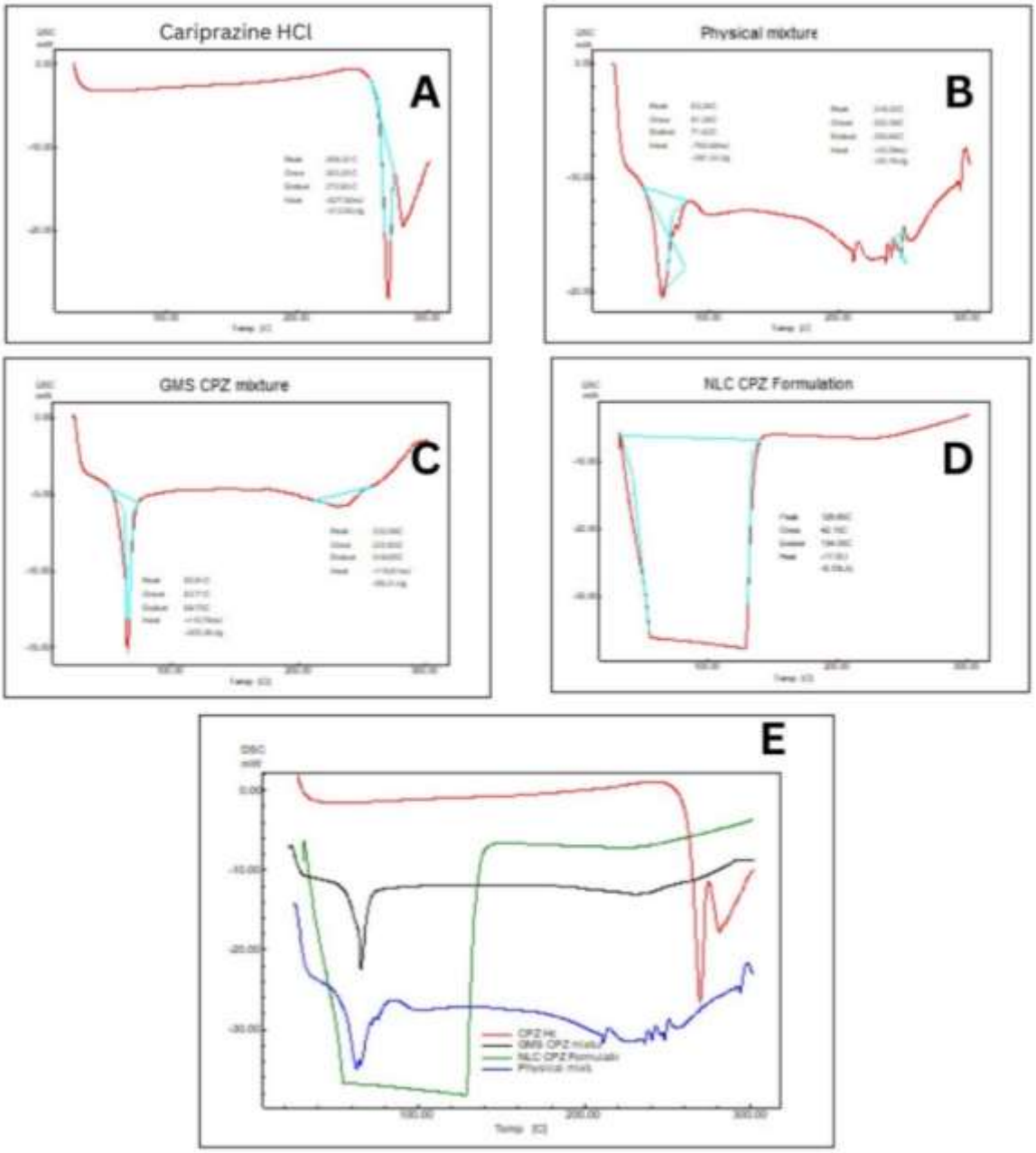


Figure 28: DSC thermogram of (A) Cariprazine hydrochloride, (B) Physical Mixture, (C) GMS and CPZ Mixture, (D) CPZ NLCs formulation, (E) Overlay

3.3.5 FTIR spectrum analysis

The spectrum of CPZ, Physical mixture and CPZ NLC are shown in Figure 29. The IR spectrum of CPZ revealed the characteristic absoption bands at 3318.82 cm^{-1} (seconary amine stretching), 2896 cm^{-1} (alkane), 1645.65 cm^{-1} (C=O secondary amine), 1442, 1349, 1178 cm^{-1} (C-H bending -CH3 Group), 1051 cm^{-1} (C-N Stretching), 954 cm^{-1} (C=C Bending),781 cm^{-1} (C-H bending), 718 cm^{-1} (C-Cl). IR spectrum of physical mixture shows absorption bands at 3341 cm^{-1} (N-H stretching amine), 2921 cm^{-1} (C-H Stretching alkane), 2862 cm^{-1} (C-H Stretching), 1733 cm^{-1} (C=O Stretching), 1456cm^{-1} (C-H Bending alkane), 1249 cm^{-1} (C-N Stretching amine), 942 cm^{-1} (C=C Bending), 850 cm^{-1} (C-Cl), 723 cm^{-1} (C-Cl Stretching). The presence of CPZ in the nanostructured lipid carrier is confirmed by the presence of 3368 cm^{-1},2922 cm^{-1},1639 cm^{-1},1459cm^{-1}, and 1096 cm^{-1} in the CPZ NLC formulation. These results indicate that there is no chemical interaction between the drug and lipid matrix. Due to changes in the chemical environment and intermolecular interactions brought on by the dispersed drug molecule in the lipid matrix, there has been a slight shifting of the CPZ absorption bands in CPZ NLC.

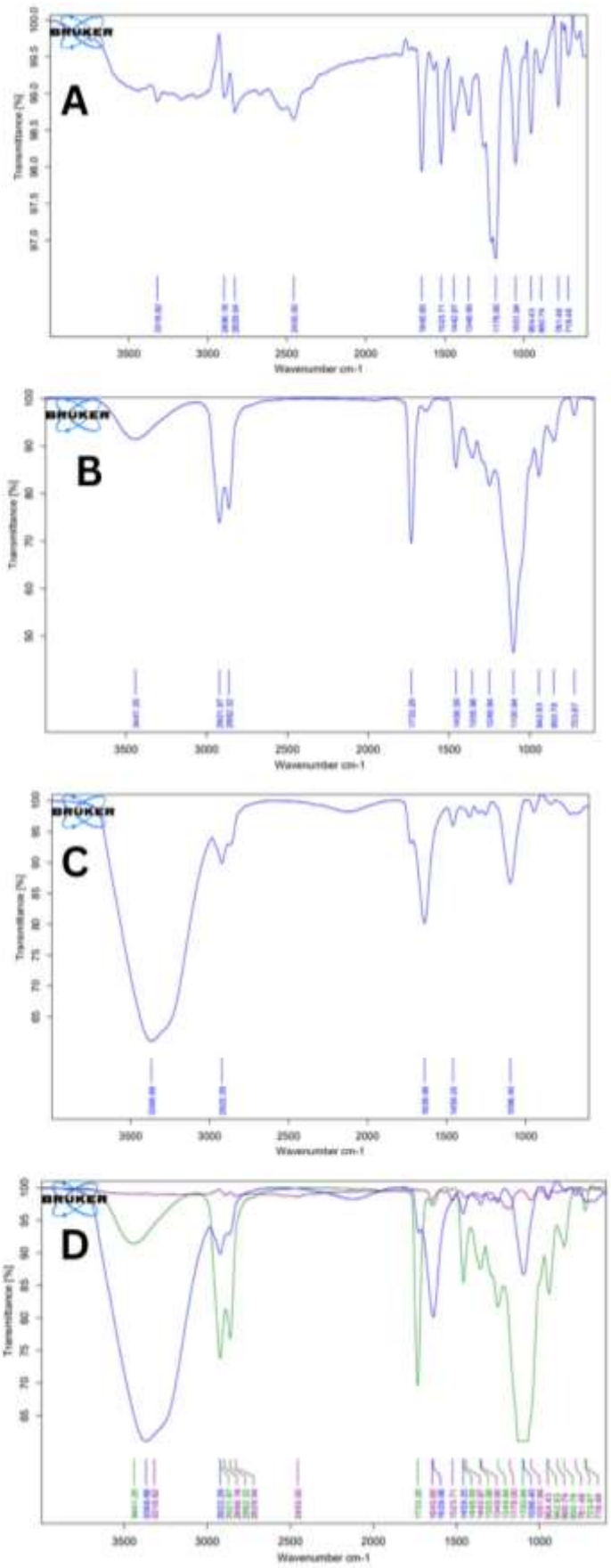


Figure 29: FTIR spectra of (A) CPZ, (B) Physical Mixture, (C) CPZ- NLC Formulation and (D) Overlay

3.3.6 *In vitro* release analysis

The in vitro drug release study utilized the dialysis membrane technique with a molecular weight cut-off of 12000-14000 Dalton. The results demonstrated a drug release of 96.11±2% for the F6 formulation, 91.23±0.57% for the F2 formulation, and 89.54±1.60% for the F8 formulation at the end of 30 minutes. The drug molecules trapped in the matrix have a shorter average diffusion path due to their smaller-sized nanosystems, which facilitates quicker diffusion and increases drug release. The in vitro data obtained for CPZ NLC were subjected to fitting with various kinetic models.

The coefficient of regression (R2) for different kinetics revealed a better fit with the first-order model, suggesting that drug release is directly proportional to the concentration gradient (58,59,69)

(F2, F6 and F8 formulations show low particle size and high entrapment efficiency among all other formulation)

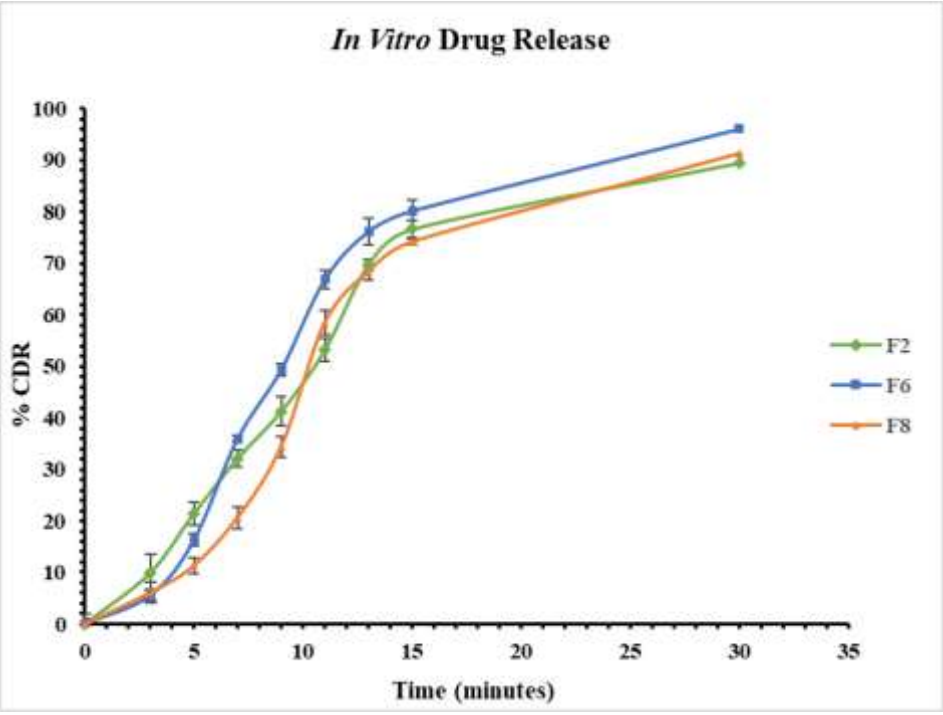


Figure 30: *In vitro* drug release of F2, F6, F8 batches

3.3.7 Study of *Ex Vivo* permeation using sheep nasal mucosa

The true characteristics of drug permeation are shown by a natural membrane. The sheep nasal mucosa was chosen for the present study due to its histological similarity to the epithelium of the human nasal mucosa. It has been demonstrated that lipid nanocarriers are essential for increasing the permeability of both hydrophilic and lipophilic drug molecules (29,70). Based on the experimental results of the permeability study, the optimized NLC formulation shows permeation of 75.83% of CPZ at the end of 35 min, whereas the CPZ suspension allowed for just 26.45%. For CPZ NLC, the steady state flux was 174.60 µg/cm^2/min, while 66.92 µg/cm^2/min was recorded for CPZ suspension. Surfactant may be the cause of the increased flow in CPZ-NLC. Increased lipophilicity of CPZ makes it much more effective in permeating through the nasal mucosa. Because of its small size and lipidic nature, CPZ NLC will be helpful in permeating drug through the olfactory epithelium. The nasal mucosa enables rapid drug delivery to the brain, ensuring a quick onset of action with drug release occurring within minutes. This contrasts with the trigeminal mucosal route, where such swift delivery is hindered by its high vascularity. Moreover, the surfactant's permeation-enhancing effect, which directly influences the cell membrane by opening pores in the nasal mucosa, promotes accelerated drug permeation.

Figure 31: Ex vivo permeation study by sheep nasal mucosa

3.3.8 Studies on Nasal Ciliotoxicity

It is essential to investigate histological alterations in the nasal mucosa induced by the formulation. No changes were seen in the nasal epithelium in the mucosa section treated with formulation CPZ-NLC. Studies on the toxicity of excipients used in the fabrication of NLCs using nasal ciliotoxicity found that nasal mucosa samples treated with PBS (negative control) did not exhibit any nasociliary damage and epithelial layers remained intact. On the other hand, severe damage to the nasal mucosa caused by IPA (Positive Control) treatment resulted in the loss of cilia, epithelial cells, and mucosal layer shrinkage (71). Hence, nasal ciliotoxicity experiments demonstrate that each excipient employed in the formulation of CPZ-loaded Nanostructured Lipid Carriers (NLCs) is deemed safe for administration via the nasal route.

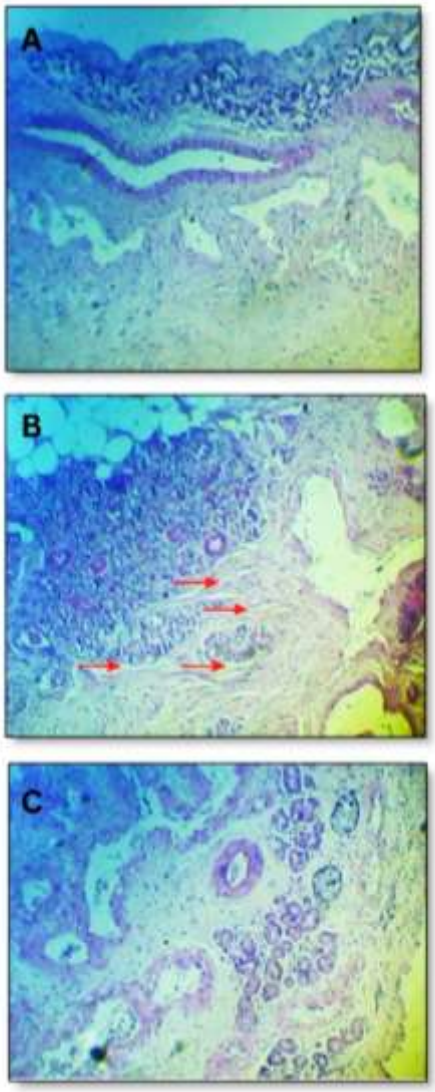


Figure 32: Histopathology section of nasal mucosa treated with (A)CPZ NLCs formulation, (B)IPA, and (C)Phosphate Buffer.

3.3.9 Drug Content

Drug loaded NLCs analyzed by developed HP-TLC method. 1 ml CPZ NLCs appropriated diluted with methanol. 0.98 ± 0.034 mg amount of CPZ was found in CPZ loaded NLC. The observation showed that the remarkably low mobile phase consumption per sample resulted in a decrease in acquisition and disposal costs. Comparing to UV spectrophotometric method which is more affordable, however HP-TLC method is more sensitive than UV method. In comparison to the HPLC method,

sample analysis is more costly and time -consuming, requiring an extended duration to complete each analysis.

3.3.10 *In vivo* Pharmacokinetic Study

During the in vivo pharmacokinetic study, the concentration of the drug in both plasma and the brain was measured at regular intervals using a validated HPLC method. The mobile phase for detection of Cariprazine was Methanol and 0.1% TFA (50:50). The linearity range for plasma was found to be 10-50 µg/ml.

The pharmacokinetic parameters were computed utilizing specialized software (Phoenix software version 8.1; Pharsight Corp, USA). Table 9 presents various pharmacokinetic parameters. The study centered on examining the distribution of CPZ within the brain after the intranasal administration of CPZ NLC, with a comparative analysis against intranasal (i.n) and oral CPZ suspension in Wistar rats.

Figure 33 illustrates the time profile of CPZ concentration in the brain after intranasal administration of CPZ NLC and CPZ drug suspension.

The concentration of CPZ in the brain achieved after intranasal (i.n.) administration of Nanostructured Lipid Carriers (NLC) 76.14± 6.23 µg/ml, T_{max} 10 min, AUC_{0-3} 450.89±28.34 was significantly higher than the oral administration of CPZ suspension (30.46±7.24 µg/ml). This can be attributed to the nanosize of carriers as well as Tween 80, and Transcutol P which enhances the permeation of NLCs. These results are in good agreement with previous research on the brain-targeting capacity of nanoparticles by tween 80(25).

Nanostructured Lipid Carriers (NLCs) have exhibited exceptional effectiveness in improving the bioavailability of CPZ within the brain. This is particularly evident when

comparing the drug concentration achieved after intranasal administration with that attained through oral administration. The concentration of the drug in the brain following intranasal delivery significantly surpasses the levels achieved through the oral route.

Moreover, the lower concentration of the drug in rat plasma following intranasal administration suggests reduced distribution to other organs in comparison to oral administration. This reduction in systemic distribution is indicative of targeted delivery facilitated by NLCs.

The consequence of this targeted delivery strategy is the minimization of peripheral effects associated with CPZ. By optimizing drug concentration in the brain while limiting systemic distribution, NLCs offer a promising approach to enhance the therapeutic efficacy of CPZ with reduced peripheral side effects.

.

Table 9: Pharmacokinetic parameters data of CPZ (Oral, Intranasal, CPZ NLC Intranasal)

Pharmacokinetic parameter		CPZ ORAL	CPZ IN	CPZ NLC IN
C max (µg/ml)	Brain	30.46±7.24	56.37± 9.72	76.14± 6.23
	Plasma	92.53±5.1	78.25±8.23	42.67±5.12
T max (min)	Brain	80±0	30±0	10±0
	Plasma	60±0	45±0	20±0
AUC 0-t(h µg/ml)	Brain	154.80±35.65	209.67±41.46	450.89±28.34
	Plasma	212.76±21.32	226.63±25.43	288.39±35.67
Bioavailability (%)	Brain	86.58	104.87	278.62
	Plasma	101.30	58.02	135.84
Half-life (h)	Brain	0.40±0.2	0.16±0.4	0.003±0.1
	Plasma	0.57±0.1	0.37±0.3	0.35±0.02
MRT(h)	Brain	0.39±0.2	0.42±0.3	0.51±0.3
	Plasma	0.55±0.04	0.57±0.13	0.33±0.15

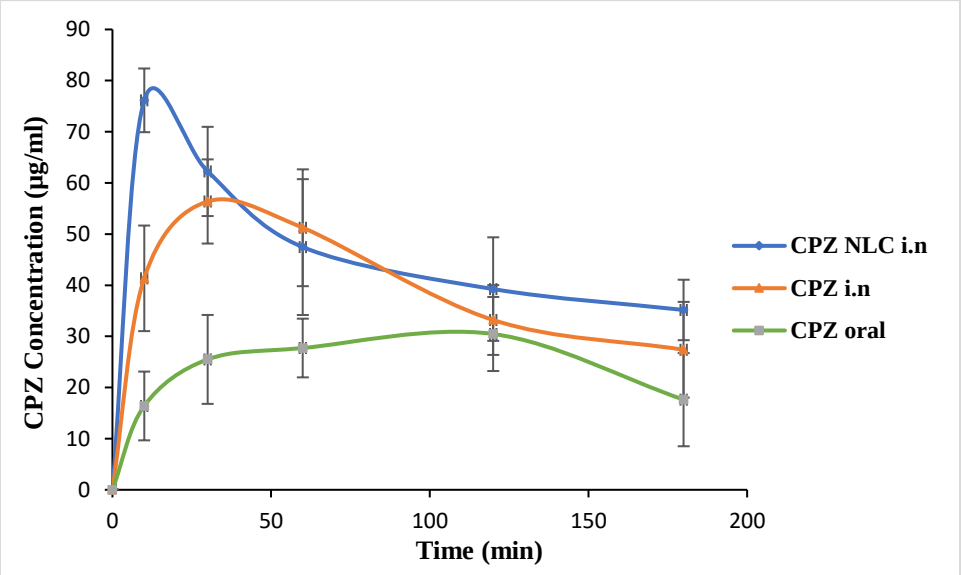


Figure 33: Brain-drug concentration-time profile of CPZ by various route

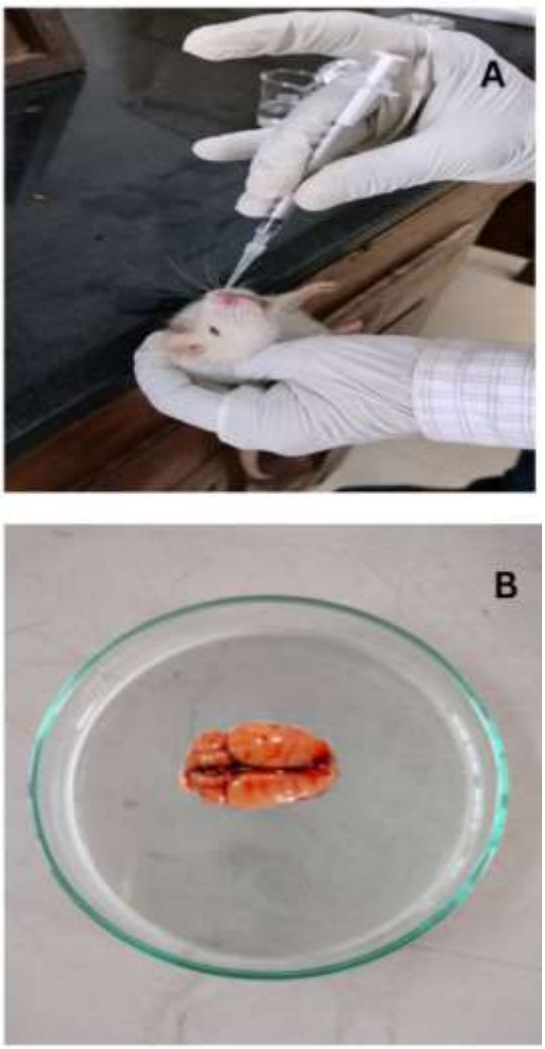


Figure 34: (A) Intranasal administration of CPZ NLCs formulation (B) Brain of rat

3.3.11 Stability Study

Stability studies for the optimized formulation (F6) were carried out, with particle size, PDI, and %EE serving as the primary parameters. The results of these studies are presented in Table 10 and Table 11. CPZ -NLCs showed no changes in color. Phase separation and caking were not observed during the stability study.

Table 10: Short term Stability study of optimized formulation F6 at room temperature (25°C/60%RH)

Parameter	0 month ± SD	1 month ± SD	2 months ± SD	3 months ± SD
Particle size (nm)	173.9±3.59	171.2±1.92	178.4±0.95	181.1±1.45
PDI	0.33±0.05	0.34±0.01	0.33±0.02	0.32±0.02
% EE	96±1.63	95.2±1.98	98.2±1.10	94.6±1.20

Table 11: Stability assessment under accelerated conditions (40°C/75%RH)

Parameter	0 month ± SD	1 month ± SD	2 months ± SD	3 months ± SD
Particle size (nm)	173.9±3.59	172.2±1.51	176.4±0.62	179.1±1.21
PDI	0.33±0.05	0.32±0.01	0.34±0.01	0.32±0.01
% EE	96±1.63	96.2±1.33	97.2±1.03	95.6±1.31

Throughout a 3-month accelerated stability study, there were no notable changes observed in the particle size, PDI, and %EE of the optimized formulation (F6). This lack of significant alterations indicates the stability of the prepared formulations.

3.4 Nasal Spray evaluation (Non metered)

pH

The pH level of the nasal formulation is crucial for maintaining normal physiological ciliary activity, preventing the growth of pathogenic microorganisms, and preventing irritation of the nasal mucosa. Nasal secretions contain lysozyme, which at acidic pH levels is responsible for eliminating specific germs. Under alkaline pH conditions, Lysozyme undergoes deactivation, rendering nasal tissue vulnerable to microbial infections. The formulation's pH was thus modified to be between 4.5 and 6.5. Digital pH meter was used to measure the pH of all developed formulations (66–68).

Clarity

The clarity of formulation was observed against white and black background visually as a translucent solution (66–68).

Viscosity

The Viscosity measurement was performed by using Brookfield Viscometer, Brookfield Engineering Laboratories LLC. The Sample was taken in a beaker and the LVT-1 spindle was used for its measurement. The spindle was dipped in to the sample and the rotation speed was carried out at 30 rpm at 25 Degree Celsius and the dial reading was noted down.

To calculate the viscosity (cps), Dial reading was multiplied with the factor. The factor for viscosity at 30 rpm and LVT-1 spindle is 2.

Content uniformity

From the prepared sample of nasal spray, 1 ml of spray solution was accurately measured and transferred to a 10 ml volumetric flask, and 5-7 ml of diluent was mixed for 2 minutes and dilutes and made up to the mark with diluent. (Concentration of Cariprazine = 100 µg/ml). % Assay was calculated using the following formula;

$$\% Assay = \frac{Ru}{Rs} \times \frac{Cs}{Cu} \times 100$$

where, Ru = Peak response of Cariprazine in the sample

Rs = Peak response of Cariprazine in the standard

Cs = Concentration of Cariprazine in standard (in µg/ml)

Cu = Concentration of Cariprazine in Sample (in µg/ml)

Table 12: Nasal spray evaluation results

Formulation	pH	Clarity	g per 10 actuations	Viscosity cps	Spray Content Uniformity %Assay
F2	5.56	Translucent	4.612	0.96	100.8
F6	5.54	Translucent	4.921	0.93	101.6
F8	5.57	Translucent	4.781	0.99	101.3

SUMMARY

Cariprazine is categorized as an atypical antipsychotic medication and is utilized in the treatment of schizophrenia. Its therapeutic effects in the treatment of schizophrenia and bipolar disorder are believed to result from a combination of partial agonism at dopamine D2 receptors, partial agonism at serotonin 5-HT1A receptors, and antagonism at serotonin 5-HT2A receptors. CPZ is a BCS Class II drug having low aqueous solubility. It shows first-pass metabolism. The most often reported side effects included akathisia, EPS, headaches, dizziness, tremors, and gastrointestinal issues. Nasal administration offers numerous benefits, including a high rate of absorption, minimum drug degradation, and a quick onset of action. In the present study formulation and characterization of the intranasal NLC of Cariprazine drug using QbD concept was developed.

HPLC and HP-TLC methods for Cariprazine were developed and validated as per ICH guidelines. Cariprazine hydrochloride-loaded NLCs was successfully formulated by melt emulsification ultrasonication method. Box Behnken design approach was used for optimization of NLCs formulations and 15 formulations were evaluated for Particle size and entrapment efficiency. The obtained findings were fitted to various polynomial models, and various statistical models were used to identify the best fit model. Thus, response surface plots produced by the design expert programme were used to illustrate how the independent and dependent variables related to one another. Glyceryl monostearate (GMS) and Capmul MCM were chosen as the solid and liquid lipids based on the drug's highest solubility. The NLCs batch was optimized based on smaller particle size and high entrapment efficiency. Zetasizer Nano ZS were used to characterize particle size, polydispersity index, and zeta potential. The surface morphology of the NLCs was confirmed by SEM and TEM analysis. The compatibility

of the drug with other excipients were studied by FTIR and DSC analysis. The in vitro drug release study was conducted employing the dialysis membrane technique, revealing a drug release of 96.11±2% for the F6 formulation. Additionally, an ex vivo permeation study was undertaken using sheep nasal mucosa. Surfactant may be the cause of the increased flow in CPZ-NLC. Increased lipophilicity of CPZ makes it much more effective in permeating through the nasal mucosa. Freshly isolated sheep mucosa was used for the nasal ciliotoxicity investigations. Nasal ciliotoxicity experiments show that every excipient used in the formulation of CPZ-loaded NLCs was safe enough to be administered via the nasal route. In the in vivo pharmacokinetic study, the drug concentration was measured in plasma and brain at regular intervals using the validated HPLC method. Nanostructured Lipid Carriers (NLCs) have shown enhanced efficacy in boosting the bioavailability of CPZ in the brain. The concentration of the drug reaching the brain following intranasal administration was significantly higher than that achieved through oral administration. Formulation was found to be stable for 3 months. Nasal spray evaluated for pH, viscosity, clarity, pump delivery (g per actuation), and content uniformity.

CONCLUSION

In the present research work, an attempt was made to improve the bioavailability of Cariprazine hydrochloride by the intranasal route. Successful preparation and optimization of cariprazine- loaded Nanostructured Lipid Carriers (NLCs) were achieved using the Box-Behnken Design.

FTIR and DSC analyses revealed that there was no interaction between the drug CPZ and the excipients used in the formulation. Cariprazine hydrochloride was shown higher solubility in methanol and was poorly water-soluble. The DSC study demonstrated the successful loading of the drug. The optimized formulation (F6) shows the desirable particle size and greater entrapment efficiency. SEM and TEM study for NLCs reveals the spherical shape of nanoparticles. In vitro drug release study for CPZ NLCs showed that drug release of 96.11± 2 % at end of 30 min. The coefficient of regression (R2) for various kinetics revealed a better fit with the first-order model, suggesting that drug release is directly proportional to the concentration gradient. Based on the experimental results of the permeability study, the optimized NLC formulation shows permeation of 75.83% of CPZ at end of 35 min. Nanostructured Lipid Carriers (NLCs) have demonstrated significant efficacy in enhancing the bioavailability of Cariprazine (CPZ) within the brain. Following intranasal administration, the drug concentration reaching the brain was notably higher compared to oral administration. Additionally, the drug concentration in rat plasma was lower, suggesting reduced distribution to other organs. This intranasal route provides non-invasive method of targeting the brain by crossing the BBB and prevent hepatic first pass metabolism as a safe and convenient drug delivery strategy for treatment of Schizophrenia's disease. Further preclinical studies required to prove effectiveness of nasal formulation in

treatment of Schizophrenia by intranasal route. To fully explore the potential of CPZ-NLCs it needs to be investigated exhaustively at clinical level.

BIBLIOGRAPHY

1. NIMH » Schizophrenia [Internet]. Available from: https://www.nimh.nih.gov/health/topics/schizophrenia

2. Coffey M. Schizophrenia: a review of current research and thinking. J Clin Nurs [Internet]. 1998 Nov 1;7(6):489–98. Available from: https://onlinelibrary.wiley.com/doi/full/10.1046/j.1365-2702.1998.00204.x

3. Psychiatry.org - What is Schizophrenia? [Internet]. Available from: https://www.psychiatry.org/patients-families/schizophrenia/what-is-schizophrenia

4. Schizophrenia, WHO. 2018.

5. Treatment - Schizophrenia - NHS [Internet]. Available from: https://www.nhs.uk/mental-health/conditions/schizophrenia/treatment/

6. Saparia P, Patel A, Shah H, Solanki K, Patel A, Sahayata M. Schizophrenia: A Systematic Review. Journal Clinical and Experimental Psychology. 2022; 2022:65–70.

7. Cariprazine | C21H32Cl2N4O - PubChem [Internet]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Cariprazine

8. Laszlovszky I, Barabássy Á, Németh G. Cariprazine, A Broad-Spectrum Antipsychotic for the Treatment of Schizophrenia: Pharmacology, Efficacy, and Safety. Adv Ther [Internet]. 2021 Jul 1;38(7):3652–73. Available from: https://link.springer.com/article/10.1007/s12325-021-01797-5

9. Stahl SM, Laredo S, Morrissette DA. Cariprazine as a treatment across the bipolar I spectrum from depression to mania: mechanism of action and review of clinical data. Ther Adv Psycho pharmacol [Internet]. 2020 [cited 2023 Dec 1];10. Available from: /pmc/articles/PMC7016327/

10. Kiss B, Horváth A, Némethy Z, Schmidt É, Laszlovszky I, Bugovics G, et al. Cariprazine (RGH-188), a dopamine D(3) receptor-preferring, D(3)/D(2) dopamine receptor antagonist-partial agonist antipsychotic candidate: in vitro and neurochemical profile. J Pharmacol Exp Ther [Internet]. 2010 Apr; 333(1):328–40. Available from: https://pubmed.ncbi.nlm.nih.gov/20093397/

11. Citrome L. Cariprazine for acute and maintenance treatment of adults with schizophrenia: an evidence-based review and place in therapy. Neuropsychiatr Dis Treat. 2018; 14:2563-2577; Available from: http://dx.doi.org/10.2147/NDT.S159704

12. Cariprazine - Mechanism of Action & Psychopharmacology [Internet]. Available from: https://psychscenehub.com/psychinsights/cariprazine-mechanism-of-action-psychopharmacology-clinical-application/

13. Behl CR, Pimplaskar HK, Sileno AP, deMeireles J, Romeo VD. Effects of physicochemical properties and other factors on systemic nasal drug delivery. Adv Drug Deliv Rev. 1998 Jan;29(1–2):89–116.

14. Tai J, Han M, Lee D, Park IH, Lee SH, Kim TH. Different Methods and Formulations of Drugs and Vaccines for Nasal Administration. Pharmaceutics. 2022 May 17;14(5):1073.

15. Chhajed S, Sangale S, Barhate SD. Advantageous nasal drug delivery system: a review. International Journal of Pharmaceutical Sciences and Research. 2011 Jun 1;2(6):1322.

16. Khosa A, Reddi S, Saha RN. Nanostructured lipid carriers for site-specific drug delivery. Biomedicine & Pharmacotherapy. 2018 Jul; 103:598–613.

17. Naseri N, Valizadeh H, Zakeri-Milani P. Solid lipid nanoparticles and nanostructured lipid carriers: Structure preparation and application. Advanced Pharmaceutical Bulletin. Tabriz University of Medical Sciences; 2015; 5, 305–13.

18. Shailendra S, Akshada A. Aghav, Devang SM, Shital SK and Mukesh PR nanostructured lipid carriers: the advanced lipid carriers and their applications. Int j pharm sci res. 2023;14(2):547-561.

19. Garg J, Pathania K, Sah SP, Pawar S V. Nanostructured lipid carriers: a promising drug carrier for targeting brain tumours. Futur J Pharm Sci [Internet]. 2022; 8:25. Available from: https://doi.org/10.1186/s43094-022-00414-8

20. Gomaa E, Fathi HA, Eissa NG, Elsabahy M. Methods for preparation of nanostructured lipid carriers. Methods. 2022 Mar; 199:3–8.

21. Cunha S, Costa CP, Moreira JN, Sousa Lobo JM, Silva AC. Using the quality by design (QbD) approach to optimize formulations of lipid nanoparticles and nanoemulsions: A review. Nanomedicine. 2020 Aug 1; 28:102206.

22. Zagalo DM, Silva BMA, Silva C, Simões S, Sousa JJ. A quality by design (QbD) approach in pharmaceutical development of lipid-based nanosystems: A systematic review. J Drug Deliv Sci Technol. 2022 Apr 1; 70:103207.

23. Alaayedi MH, Maraie NK. Lomustine nanoemulsion as nose-to-brain drug delivery system for CNS tumor treatment. Saudi Pharmaceutical Journal. 2023 Aug 1;31(8):101692.

24. Tian Z, Yi Y, Yuan H, Han J, Zhang X, Xie Y, et al. Solidification of nanostructured lipid carriers (NLCs) onto pellets by fluid-bed coating: Preparation, in vitro characterization and bioavailability in dogs. Powder Technol. 2013 Oct 1; 247:120–7.

25. Gao M, Mei D, Huo Y, Mao S. Effect of polysorbate 80 on the intranasal absorption and brain distribution of tetramethylpyrazine phosphate in rats. Drug Deliv Transl Res [Internet]. 2019 Feb 15; 9(1):311–8. Available from: https://link.springer.com/article/10.1007/s13346-018-0580-y

26. Mehmood Y, Shahid H, Abbas M, Farooq U, Alshehri S, Alam P, et al. Developing Nanosuspension Loaded with Azelastine for Potential Nasal Drug Delivery: Determination of Proinflammatory Interleukin IL-4 mRNA Expression and Industrial Scale-Up Strategy. ACS Omega. 2023 Jul 4;8(26):23812–24.

27. Jazuli I, Annu, Nabi B, Moolakkadath T, Alam T, Baboota S, et al. Optimization of Nanostructured Lipid Carriers of Lurasidone Hydrochloride Using Box-Behnken Design for Brain Targeting: In Vitro and In Vivo Studies. J Pharm Sci. 2019 Sep 1;108(9):3082–90.

28. Dadhania P, Vuddanda PR, Jain A, Velaga S, Singh S. Intranasal delivery of asenapine loaded nanostructured lipid carriers: formulation, characterization, pharmacokinetic and behavioural assessment. RSC Adv [Internet]. 2015 Dec

24; 6(3):2032–45. Available from: https://pubs.rsc.org/en/content/articlehtml/2016/ra/c5ra19793g

29. Gadhave DG, Tagalpallewar AA, Kokare CR. Agranulocytosis-Protective Olanzapine-Loaded Nanostructured Lipid Carriers Engineered for CNS Delivery: Optimization and Hematological Toxicity Studies. AAPS PharmSciTech [Internet]. 2019 Jan 1;20(1):1–15. Available from: https://link.springer.com/article/10.1208/s12249-018-1213-y

30. Sivadasu P, Gowda DV, Siddaramaiah H, Hemalatha S. Ziprasidone hydrochloride loaded nanostructured lipid carriers (NLCs) for intranasal delivery: Optimization and in vivo studies. International journal of applied pharmaceutics. 2020 Jan 15:31-41. Available from: https://innovareacademics.in/journals/index.php/ijap/article/view/35683/21425

31. Ferreira MD, Duarte J, Veiga F, Paiva-Santos AC, Pires PC. Nanosystems for Brain Targeting of Antipsychotic Drugs: An Update on the Most Promising Nanocarriers for Increased Bioavailability and Therapeutic Efficacy. Pharmaceutics [Internet]. 2023 Feb 17 ;15(2):678. Available from: https://www.mdpi.com/1999-4923/15/2/678/htm

32. Nair SC, Vinayan KP, Mangalathillam S. Nose to brain delivery of phenytoin sodium loaded nano lipid carriers: Formulation, drug release, permeation and in vivo pharmacokinetic studies. Pharmaceutics. 2021 Oct 1;13(10).

33. Chiprikar P, Mastiholimath V. Method development and validation of cariprazine hydrochloride in bulk by UV spectrophotometric method. Indian J Novel Drug Deliv. 2022; 14:52-7. Available from: https://www.ijndd.in/method-

development-and-validation-of-cariprazine-hydrochloride-in-bulk-by-uv-spectrophotometric-method/

34. Pradhan K, Mishra U, Pattnaik S, Panda C, Sahu K. Development and Validation of a Stability-indicating UV Spectroscopic Method for Candesartan in Bulk and Formulations. Indian J Pharm Sci [Internet]. 2011 Nov;73(6):693. Available from: /pmc/articles/PMC3480759/

35. Jain PS, Chaudhari AJ, Patel SA, Patel ZN, Patel DT. Development and validation of the UV-spectrophotometric method for determination of terbinafine hydrochloride in bulk and in formulation. 2011; 2, 198-202 Available from: www.phmethods.org

36. Pathak S, Mishra P. Stability-indicating HPLC-DAD method for the determination of empagliflozin. Future Journal of Pharmaceutical Sciences 2021 7:1 [Internet]. 2021 Aug 30; 7(1):1–8.

Available from: https://fjps.springeropen.com/articles/10.1186/s43094-021-00329-w

37. Shah U, Patel S, Raval M. Stability Indicating Reverse Phase HPLC Method for Estimation of Rifampicin and Piperine in Pharmaceutical Dosage Form. Curr Drug Discovery Technologies. 2017 Jun 19;15(1):54–64.

38. Kumar L, Sreenivasa Reddy M, Managuli RS, Pai K. G. Full factorial design for optimization, development and validation of HPLC method to determine valsartan in nanoparticles. Saudi Pharmaceutical Journal. 2015 Oct 1;23(5):549–55.

39. Bhadra S, Das SC, Roy S, Arefeen S, Rouf AS. Development and Validation of RP-HPLC Method for Quantitative Estimation of Vinpocetine in Pure and Pharmaceutical Dosage Forms. Chromatography Research International. 2011 Nov 15; 2011:1–7.

40. Vijayaraj S, Palei NN, Archana D, Lathasri K, Rajavel P. Quality by design approach to develop stability indicating HPLC method for estimation of rutin in chitosan-sodium alginate nanoparticles. Brazilian Journal of Pharmaceutical Sciences. 2020;56.

41. International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use ICH harmonised tripartite guideline validation of analytical procedures: text and methodology Q2(R1).

42. Hajmalek M, Goudarzi M, Ghaffari S, Attar H, Mazlaghan MG. Development and validation of a HPTLC method for analysis of Sunitinib malate. Brazilian Journal of Pharmaceutical Sciences [Internet]. 2016; 52(4). Available from: http://dx.doi.org/10.1590/S1984-82502016000400003

43. Shah NJ, Shah SJ, Patel DM, Patel NM. Development and Validation of HPTLC Method for the Estimation of Etoricoxib. Indian journal of pharmaceutical sciences. 2006 Nov;68(6):788-9.

Available from: https://www.ijpsonline.com/articles/development-and-validation-of-hptlc-method-for-the-estimation-of-etoricoxib.html

44. Sreekanth N, Awen BZ, Rao CB. HPTLC method development and validation of trandolapril in bulk and pharmaceutical dosage forms. Journal of advanced

pharmaceutical technology & research. 2010 Apr;1(2): 172.Available from: www.japtr.org

45. Patel RB, Patel MR, Bhatt KK, Patel BG. Development and validation of an HPTLC method for determination of olanzapine in formulations. Journal of AOAC International. 2010 May 1;93(3):811-9. Available from: https://academic.oup.com/jaoac/article/93/3/811/5655711

46. Shirode AR, Jadhav BG, Kadam VJ. HPTLC method development and validation of zolpidem tartrate in bulk and marketed formulation. International Journal of Pharmaceutical Sciences and Drug Research [Internet]. 2015 Mar 1;7(2):193–7.

47. Alam P, Ezzeldin E, Iqbal M, Anwer MK, Mostafa GAE, Alqarni MH, et al. Ecofriendly densitometric RP-HPTLC method for determination of rivaroxaban in nanoparticle formulations using green solvents. RSC Adv [Internet]. 2020 Jan 10; 10(4):2133–40.

48. Jain PS, Patel MK, Bari SB, Surana SJ. Development and validation of HPTLC method for simultaneous determination of amlodipine besylate and metoprolol succinate in bulk and tablets. Indian J Pharm Sci [Internet]. 2012 Mar;74(2):152. Available from: https://www.ijpsonline.com/articles/development-and-validation-of-hptlc-method-for-simultaneous-determination-of-amlodipine-besylate-and-metoprolol-succinate-in-bulk-.html

49. Deshkar SS, Jadhav MS, Shirolkar SV. Development of Carbamazepine Nanostructured Lipid Carrier Loaded Thermosensitive Gel for Intranasal Delivery. Adv Pharm Bull [Internet]. 2021 Jan 7 ;11(1):150–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/33747862

50. Anilkumar K, Kavinkumar S, Senthil V. Lipid-based nanocarrier drug delivery system for brain targeting through nasal route: a review. International journal of pharmaceutical sciences and research [Internet]. 2020; Vol. 11(10): 4774-4783 Available from: https://ijpsr.com/bft-article/lipid-based-nanocarrier-drug-delivery-system-for-brain-targeting-through-nasal-route-a-review/

51. Palagati S, Sv S, Kesavan BR. Application of computational tools for the designing of Oleuropein loaded nanostructured lipid carrier for brain targeting through nasal route. DARU Journal of Pharmaceutical Sciences. 2019 Dec;27:695-708.Available from: https://doi.org/10.1007/s40199-019-00304-0

52. Agrawal M, Saraf S, Pradhan M, Patel RJ, Singhvi G, Alexander A. Design and optimization of curcumin loaded nano lipid carrier system using Box-Behnken design. Biomedicine & Pharmacotherapy. 2021 Sep 1; 141:111919. Available from: https://doi.org/10.1016/j.biopha.2021.111919

53. Yu S, Tan G, Liu D, Yang X, Pan W. Nanostructured lipid carrier (NLC)-based novel hydrogels as potential carriers for nepafenac applied after cataract surgery for the treatment of inflammation: design, characterization and in vitro cellular inhibition and uptake studies. RSC Adv [Internet]. 2017 Mar 14;7(27):16668–77.

54. Teng Z, Yu M, Ding Y, Zhang H, Shen Y, Jiang M, et al. Preparation and characterization of nimodipine-loaded nanostructured lipid systems for enhanced solubility and bioavailability. Int J Nanomedicine [Internet]. 2018 Dec 21; 14:119–33. Available from: https://www.dovepress.com/preparation-and-characterization-of-nimodipine-loaded-nanostructured-l-peer-reviewed-fulltext-article-IJN

55. Sivadasu P, Gowda DV, Subramani NK, Vishweshwaraiah BM, Shivanna S, Hatna S. Direct brain targeted nanostructured lipid carriers for sustained release of schizophrenic drug: Formulation, characterization and pharmacokinetic studies. Indian Journal of Pharmaceutical Education and Research. 2020 Jan 1; 54(1):73–84.

56. Shah B, Khunt D, Bhatt H, Misra M, Padh H. Intranasal delivery of venlafaxine loaded nanostructured lipid carrier: Risk assessment and QbD based optimization. J Drug Deliv Sci Technol. 2016 Jun 1; 33:37–50.

57. Gokce EH, Korkmaz E, Dellera E, Sandri G, Cristina Bonferoni M, Ozer O. Resveratrol-loaded solid lipid nanoparticles versus nanostructured lipid carriers: Evaluation of antioxidant potential for dermal applications. Int J Nanomedicine [Internet]. 2012; 7:1841–50.

58. Salawi A, Almoshari Y, Sultan MH, Madkhali OA, Bakkari MA, Alshamrani M, et al. Production, Characterization, and In Vitro and In Vivo Studies of Nanoemulsions Containing St. John's Wort Plant Constituents and Their Potential for the Treatment of Depression. Pharmaceuticals. 2023 Apr 1;16(4).

59. Ali HH, Hussein AA. Oral nanoemulsions of candesartan cilexetil: Formulation, characterization and in vitro drug release studies. Aaps Open. 2017 Dec;3(1):1-6.

60. Sivadasu P, Gowda DV, Subramani NK, Vishweshwaraiah BM, Shivanna S, Hatna S. Direct Brain Targeted Nanostructured Lipid Carriers for Sustained Release of Schizophrenic Drug: Formulation, Characterization and Pharmacokinetic Studies. Indian Journal of Pharmaceutical Education and Research. 2020 Jan 1;54(1):73–84.

61. Desai GN, Dandagi PM, Kazi TM. Nanosized intranasal delivery of novel self-assembled cubic liquid crystals: formulation and evaluation. Journal of Pharmaceutical Innovation. 2023 Sep;18(3):934-51.

62. Jojo GM, Kuppusamy G, De A, Karri VN. Formulation and optimization of intranasal nanolipid carriers of pioglitazone for the repurposing in Alzheimer's disease using Box-Behnken design. Drug development and industrial pharmacy. 2019 Jul 3; 45(7):1061–72.

63. Madane RG, Mahajan HS. Curcumin-loaded nanostructured lipid carriers (NLCs) for nasal administration: design, characterization, and in vivo study. https://doi.org/103109/107175442014975382 [Internet]. 2014 May 3 ;23(4):1326–34.

64. Chiprikar P, Mastiholimath V, Balekundri A. HP-TLC Method Development of Cariprazine Hydrochloride for Applicative Quantification of Nanostructured Lipid Carriers. Chemistry Africa [Internet]. 2023 Jul 5; 1–10. Available from: https://link.springer.com/article/10.1007/s42250-023-00726-z

65. Gyertyán I, Kiss B, Sághy K, Laszy J, Szabó G, Szabados T, et al. Cariprazine (RGH-188), a potent D3/D2 dopamine receptor partial agonist, binds to dopamine D3 receptors in vivo and shows antipsychotic-like and procognitive effects in rodents. Neurochem Int. 2011 Nov 1;59(6):925–35.

66. Menaka M, Pandey VP. Formulation development and evaluation of Metoclopramide hydrochloride nasal spray. Int. J. Pharmac. Pharm. Sci. 2014;6(1):212-6.

67. Menaka M, Pandey VP. Formulation Development and Evaluation of Cinnarizine Nasal Spray. International Journal of Pharma Research and Health Sciences. 2014; 2(4):339–46. Available from: www.pharmahealthsciences.net

68. Menaka M, Pandey VP, Smith AA. Formulation development and evaluation of ondansetron hydrochloride nasal spray. International Journal of Pharmacy and Pharmaceutical Sciences. 2013; 5(4): 150-154.

69. Nair SC, Vinayan KP, Mangalathillam S. Nose to brain delivery of phenytoin sodium loaded nano lipid carriers: Formulation, drug release, permeation and in vivo pharmacokinetic studies. Pharmaceutics. 2021 Oct 1;13(10).

70. Pund S, Rasve G, Borade G. Ex vivo permeation characteristics of venlafaxine through sheep nasal mucosa. European Journal of Pharmaceutical Sciences. 2013 Jan 23;48(1–2):195–201.

71. Salade L, Wauthoz N, Goole J, Amighi K. How to characterize a nasal product. The state of the art of in vitro and ex vivo specific methods. Int J Pharm. 2019 Apr 20; 561:47–65.

KLE College of Pharmacy
A Constituent Unit of
KLE Academy of Higher Education and Research
(Deemed to be University)
DEPARTMENT OF PHARMACOLOGY
JNMC Campus, Nehru Nagar, Belagavi – 590 010, Karnataka, India
INSTITUTIONAL ANIMAL ETHICS COMMITTEE
Reg.No.221/Po/Re/S/2000/CPCSEA

DATE: 03/12/2022

CERTIFICATE

This is to certify that the project proposal no 04 entitled," Formulation and Characterisation of Nanostructured lipid carrier intra-nasal spray of cariprazine using QbD approach", submitted by Dr./ Mr. / Ms. Pallavi Chiprikar under the guidance of Dr. Vinayak Mastiholimath has been approved/recommended by the IAEC of KLE College of Pharmacy, Belagavi, Reg.No.221/Po/Re/S/2000/CPCSEA in its meeting dated 03/12/2022 , resolution No. 34 has been sanctioned ....24.... Rats/ Mice/ Rabbits/Guinea pig (animals) sex ---male--- under this proposal for a duration of next.....months.

You are hereby informed to strictly adhere to the protocol submitted for approval. Further you are required to keep the account of animals used for the project in specified Performa, Form D.

Authorized by	Name	Signature	Date
Member Secretary:	Dr. N.A. Khahb	MEMBER SECRETARY Institutional Animal Ethics Committee Reg. No. 221/PO/Rc/S/2000/CPCSEA KLE College of Pharmacy, BELAGAVI.	3/12/22
Main Nominee of CPCSEA:	Dr. Vishnu Kangralkar	CPCSEA Nominee Institutional Animal Ethics Committee Reg. No. 221/PO/Rc/S/2000/CPCSEA KLE College of Pharmacy, BELAGAVI.	3/12/22

Chemistry Africa
https://doi.org/10.1007/s42250-023-00726-z

ORIGINAL ARTICLE

HP-TLC Method Development of Cariprazine Hydrochloride for Applicative Quantification of Nanostructured Lipid Carriers

Pallavi Chiprikar[1] · **Vinayak Mastiholimath**[1] · **Amruta Balekundri**[2]

Received: 5 April 2023 / Accepted: 17 June 2023

Abstract

Purpose Cariprazine hydrochloride (CPZ) is a new atypical antipsychotic. A nanostructured lipid carrier (NLC) loaded with cariprazine hydrochloride was prepared by the emulsification solvent evaporation method. The HPTLC technique for cariprazine hydrochloride has not yet been established.

Methods A simple and rapid High-Performance Thin-Layer Chromatography (HP-TLC) was developed and validated for the quantification of cariprazine hydrochloride-loaded nanostructured lipid carrier on silica gel $60F_{254}$ plates, using toluene: methanol (7:3 V/V) as mobile phase. The analysis was performed at 253 nm. The method was validated according to the International Council for Harmonisation guidelines with respect to linearity, range, precision, accuracy, specificity, and robustness.

Results Cariprazine hydrochloride presents as a sharp band at R_f 0.64, with a good linear relationship with a concentration range of 1–5 µL/band with a regression coefficient of 0.993. The limit of detection and limit of quantification was found to be 0.50 µg/band and 1.52 µg/band respectively. The method was found to be precise and reliable.

Conclusion The technique is appropriate for the routine examination of cariprazine in dosage forms and in bulk. The analysis method was found to be simple, accurate, and economical.

Keywords HP-TLC · Cariprazine · Validation · Nanostructured lipid carrier

Abbreviations

CPZ	Cariprazine
HCL	Hydrochloride
RP	Reverse phase
HP-TLC	High-performance thin layer chromatography
HPLC	High-performance liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
Rf	Retention factor
SD	Standard deviation
RSD	Relative standard deviation
ICH	International Conference on Harmonization
AVG	Average
RPM	Revolution per minute

1 Introduction

A person with schizophrenia suffers from a severe mental illness that affects their thoughts, feelings, and behaviour. Schizophrenia patients may appear to have lost all sense of reality, which can be upsetting to both them and their loved ones. Participating in regular, everyday activities may be challenging for someone with schizophrenia, but there are effective therapies available. Many people who undergo treatment are able to participate in their studies or careers, grow into independent people, and cherish their relationships with others [1]. Schizophrenia affects approximately 24 million people or 1 in 300 people (0.32%) worldwide. This rate is 1 in 222 people (0.45%) among adults [2].

Cariprazine hydrochloride (CPZ) is a new atypical antipsychotic used to treat schizophrenia. It is chemically 3-[4-[2-[4-(2,3-dichlorophenyl) piperazin-1-yl]ethyl] cyclohexyl]-1,1-dimethylurea (Fig. 1).

✉ Vinayak Mastiholimath
mastiholimath@rediffmail.com

[1] Department of Pharmaceutical Quality Assurance, KLE College of Pharmacy, KLE Academy of Higher Education and Research (KAHER) Belagavi, Belagavi, Karnataka, India

[2] Department of Pharmacognosy, KLE College of Pharmacy, KLE Academy of Higher Education and Research (KAHER) Belagavi, Belagavi, Karnataka, India

Published online: 05 July 2023

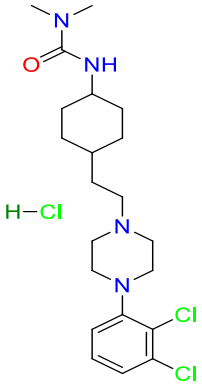


Fig. 1 Structure of Cariprazine Hydrochloride

The oral atypical antipsychotic cariprazine was developed by Gedeon Richter (Vraylar). It binds primarily to the D3 dopamine receptor and is a potent partial agonist for both D3 and D2 dopamine receptors. Moreover, cariprazine exhibits a weak agonist effect at serotonin 5-HT1A receptors. In the USA, cariprazine received its first global approval in September 2015 for the treatment of schizophrenia and the acute management of manic or mixed episodes associated with bipolar I disorder [3]. Treatment with cariprazine mostly results in D3 receptor blockage. Cariprazine has a reasonably high affinity for 5HT1A receptors, similar to many other antipsychotics, but a lower relative affinity for 5HT2A receptors. The half-life of cariprazine, the longest among all atypical antipsychotics, is 2–4 days, whereas that of its active metabolite is 1–3 weeks [4].

NLCs are modified solid lipid nanoparticles in which the lipid phase contains both solid and liquid lipid at ambient temperature which are more stable and drug-loading capacity is more [5].

High-performance thin layer chromatography (HP-TLC) has become a key technique for the analysis of numerous drugs. It offers many benefits, including low operating costs and high sample performance, and is currently a standard analytical technique. The main advantage of HP-TLC is that in contrast to high-performance liquid chromatography (HPLC), it uses a little amount of mobile phase to run several samples, lowering the cost per study and processing time.

1.1 Advantages of HP-TLC Method

No specialized person is needed; technically simple to understand and operate. There are affordable precoated HP-TLC plates available. The whole spectrum is seen at a glance. Low maintenance costs due to negligible wear and tear. It is easy to prepare samples. Solvents don't require any prior processing, such as filtering and degassing. The extremely low mobile phase consumption per sample lowers the cost of acquisition and disposal. Analytical-grade solvents are acceptable. No further purification is required. As new stationary phase and mobile phase are utilised for each study, there is no chance of interference from earlier analyses. The amount of solvent needed on an absolute basis for each sample is fairly small, which reduces the disposal issue.

Different analytical methods were reported regarding cariprazine, including the spectrophotometric method, and HPLC method. Comparing the quantitative determination of drugs using statistical methods reveals that the HPLC/HP-TLC approach is more accurate and precise than the UV method. Tiwari et al. analyzed cariprazine using a C-18 Inertsil ODS-3 (250 × 4.6 mm, 5 µm) column with a mobile phase combination of 0.05 M ammonium acetate buffer and acetonitrile (50:50 v/v) [6]. Ghumare Vaibhav et al. used chemsilods C-18 ((250 × 4.6 mm, 5 µm) column with mobile phase Acetonitrile and Ammonium Acetate Buffer (pH 4.8) in the ratio 60:40 v/v [7]. Pallekona Sushma et al. developed a method for cariprazine and its degradant using a waters C18 (150 × 4.6 mm, 3.35-micron) column with a flow rate of 1mL/min with a mobile phase of methanol and 0.1% orthophosphoric acid in the proportion of 50:50 (% v/v) [8]. Toujani E et al. analyzed cariprazine using Acetonitrile: Potassium dihydrogen orthophosphate buffer (pH 4; 50 mM) (30:70 v/v) [9].

There is no HP-TLC method for the estimation of cariprazine Hydrochloride in bulk or in a pharmaceutical formulation, according to the literature review. This is a new method developed for the estimation of cariprazine hydrochloride. The goal of the current work was to develop and validate a new, easy, accurate, precise, and selective HP-TLC method for the estimation of CPZ in accordance with ICH requirements.

2 Experimental

2.1 Materials and Reagents

Cariprazine hydrochloride was a gift sample from MSN Labs, Hyderabad. Methanol and toluene were purchased by Merck India Ltd. Capmul MCM received as a gift sample from ABITEC, USA. Glyceryl monostearate was purchased by Ozone International, Mumbai.

2.2 Instrumentation and Chromatographic Conditions

The HP-TLC system (CAMAG®, Switzerland) consisted of a TLC scanner with Linomat 5 auto sprayer connected to a nitrogen cylinder, a visualizer, and a twin trough chamber. Software called Vision CATS version 3 was utilized. TLC-Hamilton® glass syringe (Hamilton-Bonaduz Schweiz, Camag, Switzerland) Pre-coated silica gel $60F_{254}$ HP-TLC plates (10 × 10 and 20 × 10 cm, layer thickness 0.1 mm E

Merck KGaA, Darmstadt, Germany) was used as stationary phase. Samples were applied under a continuous drying stream of nitrogen gas. Development was carried out in a twin trough chamber (10 × 10 and 20 × 10 cm). The optimized chamber saturation time for the mobile phase was 10 min. The source of radiation utilized was a deuterium lamp. The bands were analyzed at a wavelength of 254 nm. Throughout experiment digital analytical balance (Sartorius), Ultra-Turrax T18 homogenizer (IKA, Wilmington, NC, USA). Probe sonicator (Sonics Pvt. Ltd., India), Sonicator (RC systems) were used.

2.3 Development of NLC

NLCs of CPZ was prepared by emulsification solvent evaporation method [10–12]. The ratio of drug to lipid was 1:10. CPZ was dissolved in 2 ml ethanol and then mixed with melted lipid (Glyceryl monostearate as solid and Capmul MCM liquid lipid in ratio of 5:5). The 3% tween 80 was prepared in distilled water in a another beaker. The lipid phase was added dropwise to the aqueous phase with constant stirring for 1 h on a magnetic stirrer. The pre-emulsion was homogenized for 15 min at 11 RPM. The emulsion so obtained was probe sonicated for 10 min (20% amplitude 10:10 on: off cycle).

2.4 Characterization of Prepared NLC

2.4.1 Particle size, Polydispersity Index, and zeta Potential

The particle size and polydispersity index were determined using zeta nano ZS (Malvern Instruments Ltd). The Zeta potential of prepared NLC was measured at 25 °C after appropriate dilution [13, 14].

2.4.2 Entrapment Efficiency

The amount of CPZ loaded to the lipid phase and its entrapment efficiency was determined by centrifugation a fixed volume of desired NLC for 30 min at 15,000 rpm. The supernatant so obtained was diluted appropriately and the amount of CPZ entrapped was quantified using UV spectrophotometric measurement at 252 nm (UV 1900, Shimadzu, Japan) using the following equation [15].

$$EE = \frac{Wd - Ws}{Wd}$$

where EE is entrapment efficiency, Wd is Total weight of the drug taken and Ws is the weight of the drug in the supernatant.

2.4.3 Transmission electron Microscopy and Scanning electron Microscopy

The morphology of formulation can be studied with SEM (Scanning electron microscopy) and TEM (Transmission electron microscopy). The surface morphology, shape, and size of the particle are all revealed by SEM. TEM provides insight into particle structure from the inside and provides information on particle diameter and matrix structure [13].

2.5 Preparation of Standard Solution and Sample

The cariprazine hydrochloride (10 mg) and cariprazine-loaded NLCs were put into two separate 10 ml volumetric flasks, dissolved, and diluted with methanol to the appropriate volume. To achieve complete drug dissolution, for 15 min solutions were placed in an ultrasonic bath and then filtered through a 45 µm membrane prior to analysis. Working standard solution of 1000 µg/ml of std drug and drug-loaded NLCs as a sample.

2.6 Method Development by HP-TLC Chromatography

Reference standard solutions of CPZ in various concentrations were made and spotted as bands on 60 F254 plates using a Camag Linomat 5 sample applicator with a bandwidth of 6 mm and a micro syringe. For plate development, a twin trough glass chamber was used. Before developing the plate in the chamber, the mobile phase was allowed to saturate for 10 min. For the solvent system, for about 70 mm, the plate was allowed to run. After the plate has reached the 70 mm distance, with the help of an air dryer, the plate is allowed to dry. When the plate is completely dry, it is analyzed using the TLC scanner 4 and the chromatograms were used to interpret the results. UV-Range Scanners are used to identify the spectra.

2.7 Analytical Method Validation

The developed method was validated as per ICH Q2 (R1) guidelines and the different parameters evaluated were linearity, range, precision, accuracy, specificity, quantification limits, and robustness [16–18].

2.7.1 Linearity and Range

For CPZ, the calibration plot's linear regression data shows an excellent linear relationship between area and concentration over the range of 1 to 5 µL/band. Peak area

versus concentration was shown on the standard calibration graph.

2.7.2 Detection Limit and Quantification Limit

A limit of detection (LOD) and a limit of quantification (LOQ) were calculated using statistical principles. According to the formula:

$$LOD = 3.3\sigma/s$$

$$LOQ = 10\sigma/s$$

where 'σ' is the standard deviation and 's' is the slope of the calibration curve.

2.7.3 Precision

The method's precision was determined by repeatability (intra-day precision) and intermediate precision (inter-day). Repeatability was determined by performing six repeated analysis of the same working solution of Cariprazine hydrochloride (3 µL/band) on the same day, under the same experimental conditions. The intermediate precision of the method was assessed by carrying out the analysis on different days. The results are shown in terms of % RSD [19, 20].

2.7.4 Accuracy

This HPTLC method's accuracy (or recovery parameter) was evaluated by using a standard drug solution at concentrations of 80%, 100%, and 120% of the target concentration (using the standard addition method). The method was carried out in triplicate based on the specific concentrations, and its mean results were expressed as a percent recovery [21].

2.7.5 Specificity

The specificity of the proposed HP-TLC method was determined by the complete separation of peaks of both formulation and API. The band of CPZ in the sample was confirmed by seeing the R_f and spectra of the standard band. The peak purity of CPZ was accessed by comparing their respective spectra. The method was therefore considered to be specific.

2.7.6 Robustness

The chromatographic conditions were changed to test the method's robustness. The choice of chromatographic conditions involves changing the composition of the mobile phase and the saturation time. Each condition was changed independently during robustness testing, while the other conditions were maintained at their optimized values.

Table 1 NLC Characterization result

Characterization parameter	Result
Particle size ± SD (nm)	180 ± 6.15
Polydispersity index ± SD	0.27 ± 0.07
Zeta potential ± SD (mV)	6.42 ± 0.84
Entrapment efficiency	87.2 ± 3.4

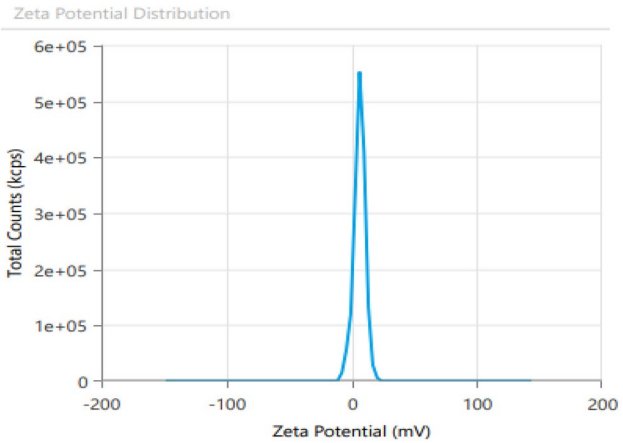


Fig. 2 Zeta potential distribution of CPZ NLCs

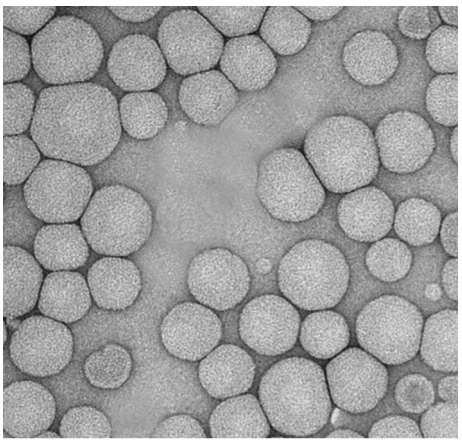


Fig. 3 SEM image of CPZ NLCs

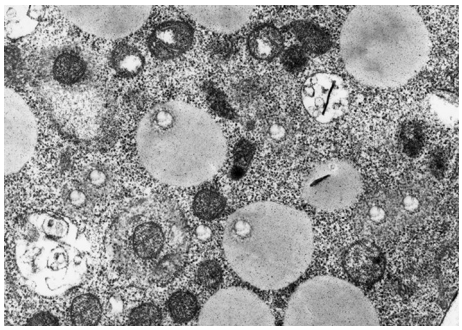


Fig. 4 TEM image of CPZ NLCs

3 Results

3.1 NLC Characterization

NLC of CPZ was prepared by emulsification solvent evaporation method. After preparation of NLCs stored in a refrigerator, samples were taken out at the time of analysis. Mean particle size and zeta potential were found to be 180 nm and 6.42 mV respectively as shown in Table 1. Figures 2, 3, and 4 shows the further characterization results of zeta potential distribution, SEM image of NLC, and TEM image of NLC. Particle size of NLCs is a crucial factor because it affects drug absorption. The smaller particle size provides larger surface area for drug absorption. Formulation with zeta potential in the range of − 30 to + 30 mV is considered as stable formulation. The particles were more homogenous as the polydispersity index value near zero. Higher polydispersity index values were the result of increasing the amount of drug and solid lipid. The proportion of drug incorporated in the lipid matrix (Entrapment efficiency) was determined. The appropriate mixture of solid and liquid lipids determines how well the drug is entrapped in the lipid matrix. SEM, TEM images revealed that NLCs were spherical in shape.

3.2 Method Optimization for the HP-TLC

In this study, different mixtures of various solvents were tried and the composition of the mobile phase with a chromatographic result having acceptable and reproducible R_f

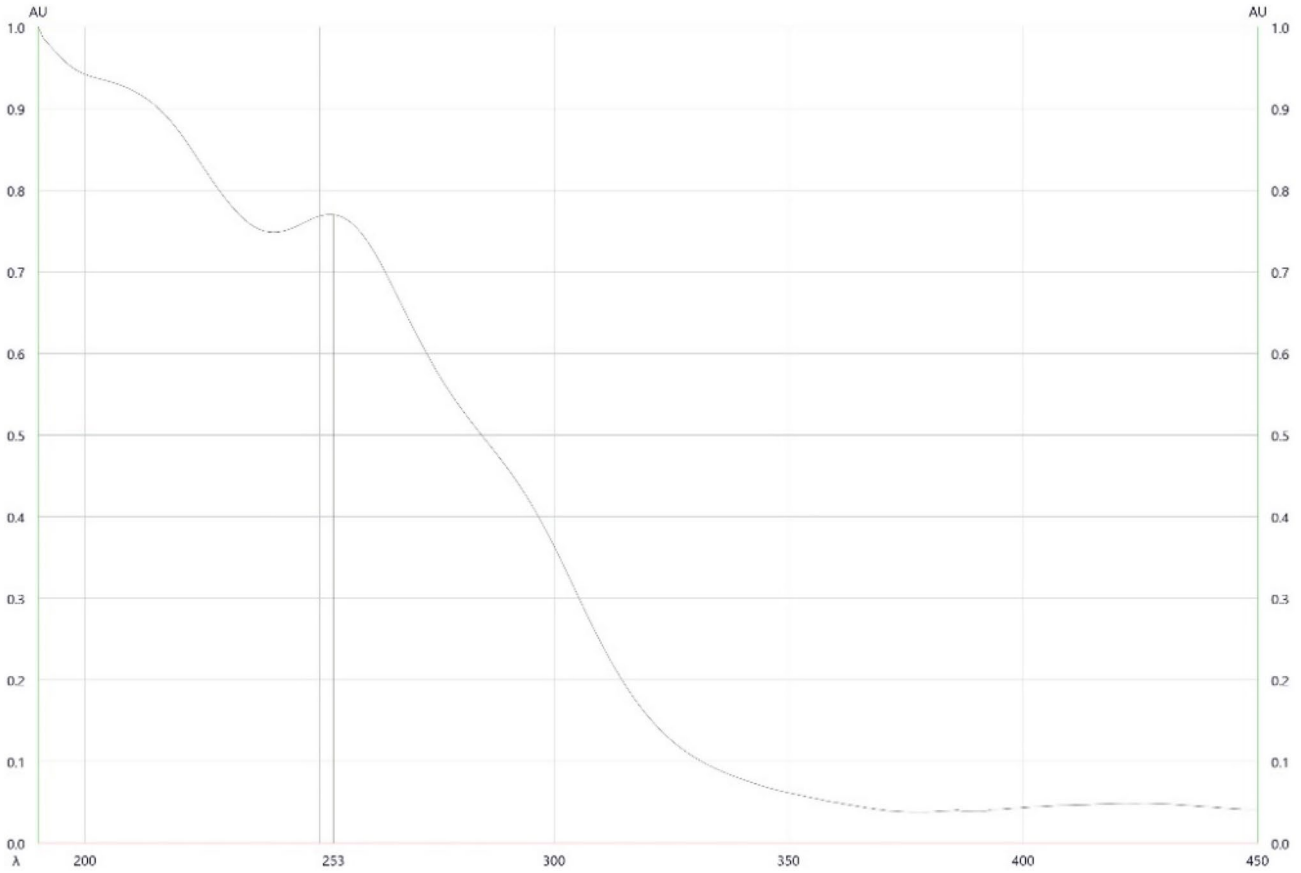


Fig. 5 Spectra of CPZ

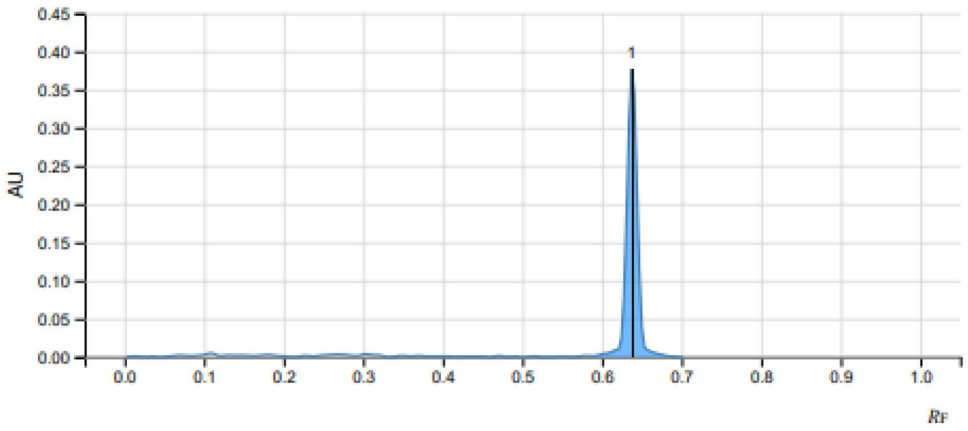


Fig. 6 Typical chromatogram of CPZ

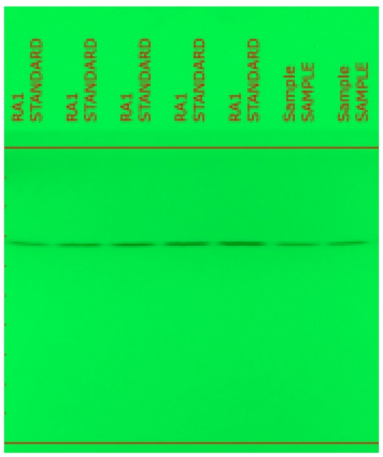


Fig. 7 Developed plate of linearity

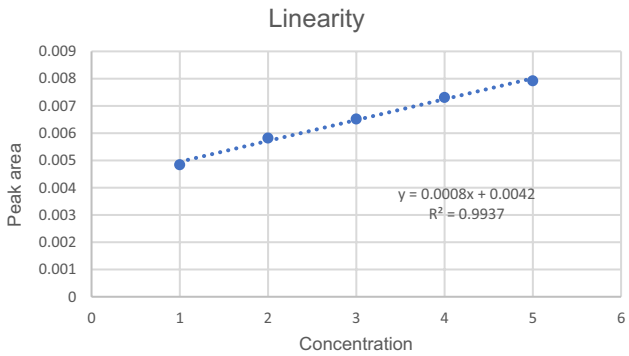


Fig. 8 Linear regression of CPZ standards

value was selected. After many trials, the optimized condition that offered a good peak was finalized. The toluene: methanol (7:3 v/v) was selected as a mobile phase. The chamber saturation time of 10 min was given. The validation of the method was performed in accordance with ICH Q2 (R1) guidelines. A wavelength of 253 nm was selected for analysis where Cariprazine hydrochloride showed higher absorbance (Fig. 5). A typical chromatogram obtained was shown in Fig. 6.

3.3 Method Validation Results

3.3.1 Linearity and Range

Cariprazine hydrochloride presents as a sharp band at R_f 0.64. In the evaluation of linearity, the peak area showed a good linear relationship with a concentration range of 1–5 µL/band. Figure 7 shows the image of the HP-TLC developed plate of linearity (Track 1–5, 1–5 µL /band). The linear regression equation was Y = 0.0008X + 0.0042, R2 = 0.9937 as shown in Fig. 8. 3D view of linearity chromatogram as seen in Vision CATS software shown in Fig. 9.

3.4 Detection Limit and Quantification Limit

The LOD and LOQ values for CPZ were found to be 0.50 and 1.52 µg/band respectively.

3.4.1 Precision

The % RSD for intraday and interday (n = 6) was found to be 0.24 and 0.23 which is less than 2% indicating the precision of the method. Table 2; Fig. 10 show the precision data for the method. The results showed that the RSD% had not exceeded 2% for any concentration, proving that all of the suggested procedures could be considered as precise method.

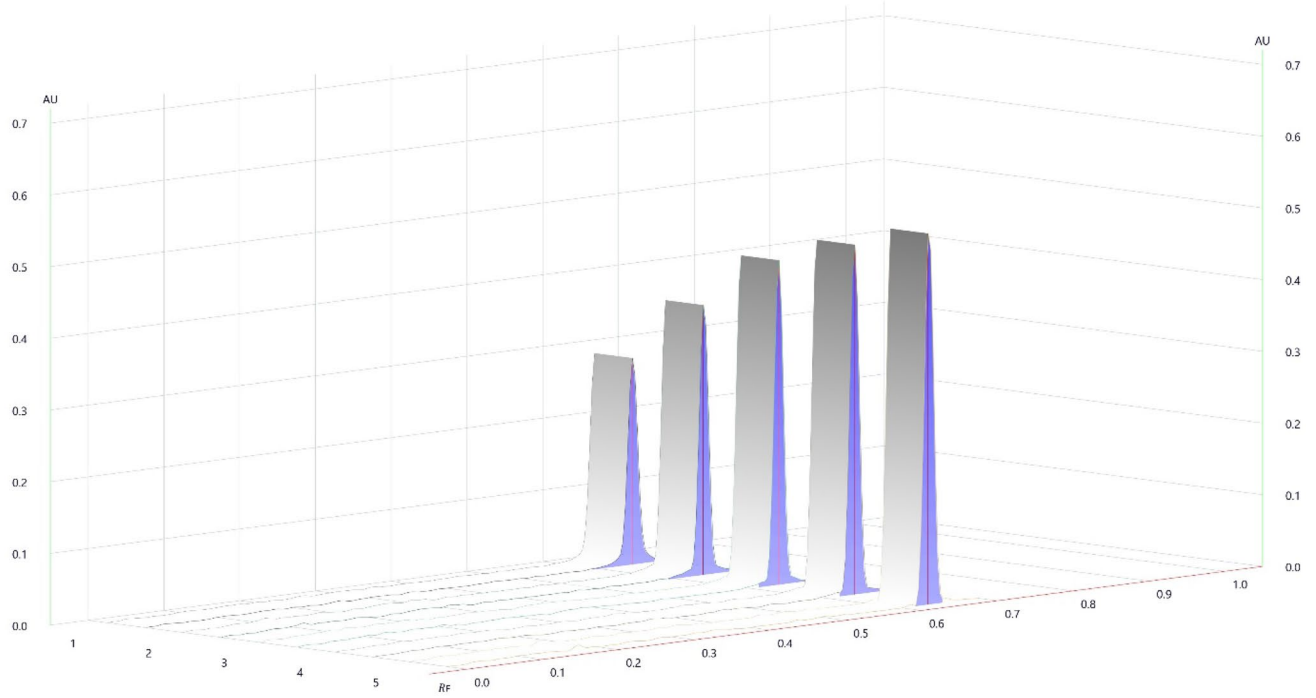


Fig. 9 3D view of Linearity chromatograms of CPZ (1,2,3,4,5 µL/band TRACK 1–5)

Table 2 Precision data

Conc µL/band	Intra-day	Inter-day		
	Peak area	Day 1 peak area	Day 2 peak area	Day 3 peak area
3	0.00729	0.00729	0.00726	0.00728
3	0.00727	0.00728	0.00730	0.00731
3	0.00731	0.00731	0.00727	0.00729
3	0.00727	0.00726	0.00725	0.00728
3	0.00729	0.00729	0.00729	0.00731
3	0.00731	0.00730	0.00729	0.00726
Mean	0.00729	0.00729	0.00729	0.00728
SD	1.78	1.72	1.97	1.94
%RSD	0.24	0.23	0.27	0.26

3.4.2 Accuracy

At 80%, 100%, and 120% levels, the determined % RSD values for cariprazine hydrochloride were found to be 1.48, 0.68, and 0.72. These were all below the 2% (acceptance limit). Results are shown in Table 3. Overall, the method demonstrated the accuracy of the established data.

3.4.3 Specificity

The complete separation of the peaks of both the NLC-loaded CPZ and API served as a measure for the suggested HP-TLC method's specificity. The R_f and spectra of the standard band confirmed the presence of the band of CPZ in the sample. The spectra of the standard and sample are depicted in Figs. 11 and 12, respectively. By comparing their individual spectra at the peak start, apex, and peak end positions of the spot, the peak purity of CPZ was determined. Hence, it was concluded that the method was specific.

3.5 Robustness

Changes in mobile phase volume and saturation duration showed percent RSD within 2%, confirming the robustness of the developed method. Table 4 depicts data of robustness.

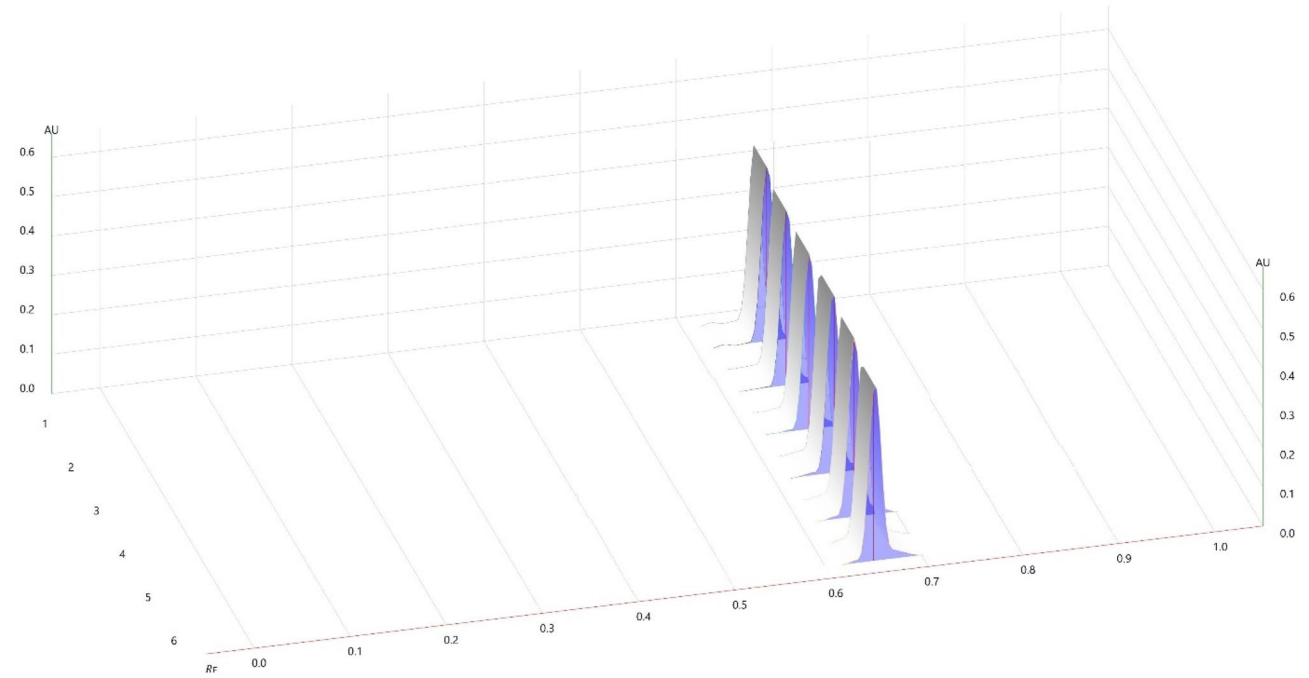


Fig. 10 3D view of Precision chromatograms of the developed method

Table 3 Results of Accuracy

% Level	Concentration (ng/band)	Area of sample	Area of standard	% Recovery	SD	%RSD
80%	2400	0.00585	0.00574	101.91	1.50	1.48
		0.00586	0.00575	101.91		
		0.0574	0.00578	99.30		
100%	3000	0.00725	0.00721	100.55	0.681	0.68
		0.00720	0.00724	99.44		
		0.00722	0.00727	99.31		
120%	3600	0.00875	0.00869	100.69	0.068	0.06
		0.00874	0.00867	100.80		
		0.00872	0.00865	100.80		

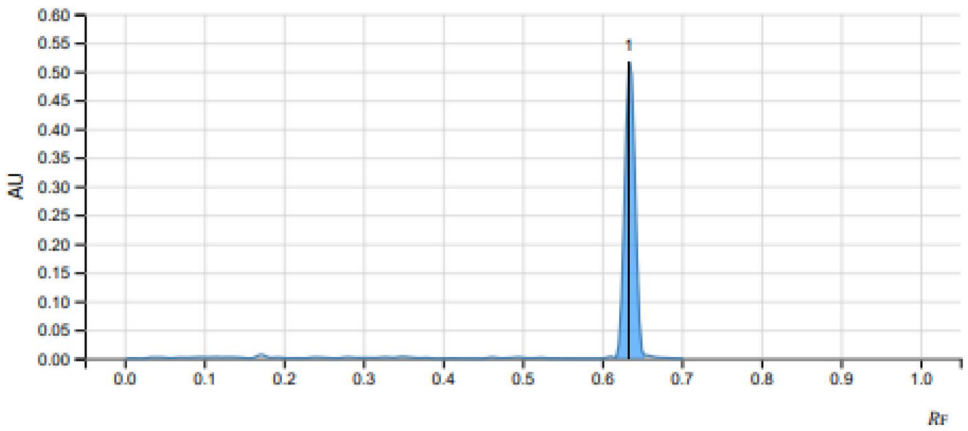


Fig. 11 Chromatogram of CPZ in bulk at 253

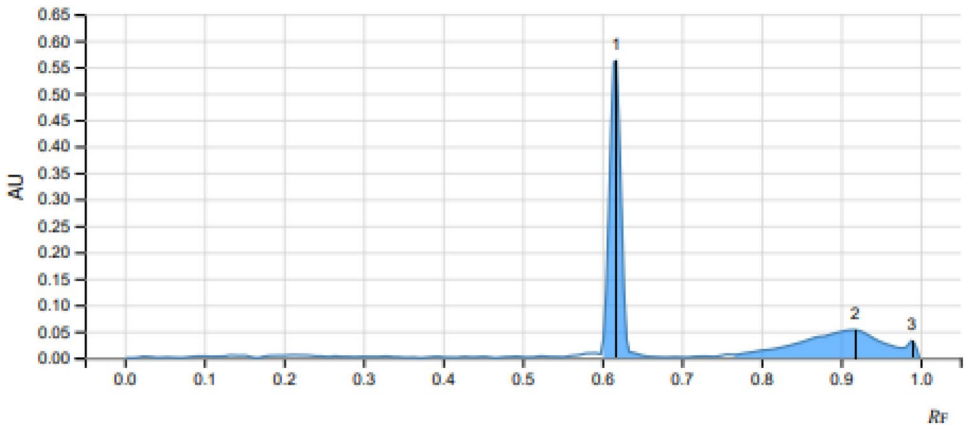


Fig. 12 Chromatogram of CPZ in the NLC at 253

Table 4 Robustness data

Factors	Chromatographic Modifications	
	Level	R_f value
Mobile phase composition (Toluene: Methanol)	±0.1ml	
	+0.1	0.644
	0	0.652
	−0.1	0.641
%RSD		0.719
Mobile phase volume	±1ml	
21	+1	0.642
20	0	0.646
19	−1	0.644
%RSD		0.253
Duration of chamber saturation	±2 min	
8	+2	0.621
10	0	0.646
12	−2	0.648
%RSD		1.924

Table 5 NLCs loaded CPZ quantification

Trials	Quantification of CPZ (mg)
1	9.91
2	9.89
3	9.87
Mean ± SD	9.89 ± 0.016

3.6 Quantification

Drug-loaded NLC was quantified in triplicate. The results were shown in Table 5 with the mean and standard deviation.

4 Discussion

The cost of acquisition and disposal is reduced by the extremely low mobile phase consumption per sample in case of HP-TLC. Comparing statistical methods for quantitative drug determination demonstrates that the HP-TLC approach is more accurate and precise than the UV technique. When comparing UV spectrophotometric method to HP-TLC, the spectrophotometric approach is shown to be more affordable, however HPTLC is more sensitive than UV spectrophotometric method [18]. Compared to HP-TLC sample analysis, HPLC sample analysis is more expensive and takes longer time to complete each sample analysis. Additionally, many samples are simultaneously analysed in a short period of time in HP-TLC analysis.

5 Conclusion

A new HP-TLC method was developed for the analysis of cariprazine hydrochloride in bulk drug. This technique was validated in accordance with ICH recommendations and proved to be repeatable, precise, accurate, and robust. As compared to other analytical techniques, the approach is quick, easy, and reasonably affordable. It is also reasonably viable, hence it is recommended for the regular analysis of cariprazine hydrochloride.

Acknowledgements The authors are also thankful to MSN Labs Hyderabad and ABITEC USA for providing gift samples of Cariprazine hydrochloride and Capmul MCM respectively.

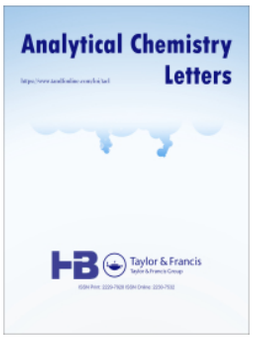
Data availability Data availability is not applicable.

Declarations

Conflict of interest The authors have no conflicts of interest regarding this investigation.

References

1. Schizophrenia. National institute of mental health (NIMH) U.S. Department of Health and Human Services. https://www.nimh.nih.gov/health/topics/schizophrenia. Accessed 25 Feb 2023
2. Schizophrenia (2023) World health Organisation (WHO). https://www.who.int/news-room/fact-sheets/detail/schizophrenia. Accessed 25 Feb 2023
3. McCormack PL (2015) Cariprazine: first global approval. Drugs. https://doi.org/10.1007/S40265-015-0494-7
4. Stahl SM (2016) Mechanism of action of cariprazine. CNS Spectrums 21(2):123–127. https://doi.org/10.1017/S1092852916000043
5. Naseri N, Valizadeh H, Zakeri-Milani P (2015) Solid lipid nanoparticles and nanostructured lipid carriers: structure preparation and application. Adv Pharm Bull 5:305–313
6. Tiwari P, Hamrapurkar PD (2020) Development and validation of a stability indicating RP-HPLC method for determination of cariprazine in bulk drug. World J Pharm Res 084:741–753. https://doi.org/10.20959/wjpr20204-16991
7. Ghumare V, Lahu H, Mhaske M, Dhonde (2022) Development and validation of analytical method for estimation of cariprazine hydrochloride in bulk and tablet dosage form by using RP-HPLC method. Int J Pharm Res Appl 7:2280–2294
8. Sushma P, Pawar AK (2022) Identification, separation and mass spectral characterization of degradants in Cariprazine HCl by LC-MS/MS/QTOF. J Chem Metrol 1:1307–6183. https://doi.org/10.25135/jcm.68.2201.2338
9. Toujani E, Mejri W, Lassoued HE, Toujani S, Fliss O, Cheikh MHB, Safta F (2023) Development and validation of a stability-indicating high-performance liquid chromatographic assay for determination of cariprazine in bulk form and in drug product. Ann Pharm Fr 81:83–93. https://doi.org/10.1016/J.PHARMA.2022.08.007
10. Jazuli I, Annu, Nabi B, Moolakkadath T, Alam T, Baboota S, Ali J (2019) Optimization of nanostructured lipid carriers of lurasidone hydrochloride using Box-Behnken design for brain targeting: in vitro and in vivo studies. J Pharm Sci 108:3082–3090. https://doi.org/10.1016/j.xphs.2019.05.001
11. Dange SM, Kamble MS, Bhalerao KK, Chaudhari PD, Bhosale AV, Nanjwade BK, Shinde SA (2014) Formulation and evaluation of venlafaxine nanostructured lipid carriers. J Bionanosci 8:81–89. https://doi.org/10.1166/jbns.2014.1209
12. Tripathi D, Sonar PK, Parashar P, Chaudhary SK, Upadhyay S, Saraf SK (2021) Augmented brain delivery of cinnarizine through nanostructured lipid carriers loaded in situ gel: in vitro and pharmacokinetic evaluation. BioNanoScience. https://doi.org/10.1007/s12668-020-00821-2/Published
13. Salvi VR, Pawar P (2019) Nanostructured lipid carriers (NLC) system: a novel drug targeting carrier. J Drug Deliv Sci Technol 51:255–267. https://doi.org/10.1016/j.jddst.2019.02.017
14. Bahari LAS, Hamishehkar H (2016) The impact of variables on particle size of solid lipid nanoparticles and nanostructured lipid carriers; a comparative literature review. Adv Pharm Bull 6:143–151. https://doi.org/10.15171/apb.2016.021
15. Sivadasu P, Gowda DV, Subramani NK, Vishweshwaraiah BM, Shivanna S, Hatna S (2020) Direct brain targeted nanostructured lipid carriers for sustained release of schizophrenic drug: Formulation, characterization and pharmacokinetic studies. Indian J Pharm Educ Res 54:73–84. https://doi.org/10.5530/IJPER.54.1.9
16. International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use ICH harmonised tripartite guideline validation of analytical procedures: text and methodology Q2(R1) Current Step 4 version 2005. https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf. Accessed 27 Sept 2022
17. Balekundri A, Mannur VK, Chouhan M (2022) A simple and validated HP-TLC method for simultaneous analysis of ethnomedicine gallic acid and eugenol. Indian Drugs 59:82–87. https://doi.org/10.53879/ID.59.09.12346
18. Chiprikar P, Mastiholimath V (2022) Method development and validation of cariprazine hydrochloride in bulk by UV §spectrophotometric method. Indian J Novel Drug Deliv 14:52–57
19. Ravi Sankar P, Anusha S (2019) Development and validation of RP-HPLC method for the determination of dasatinib in tablet dosage form. Int J Pharm Sci Res 10:4531. https://doi.org/10.13040/IJPSR.0975-8232.10(10).4531-37
20. Bhadra S, Das SC, Roy S, Arefeen S, Rouf ASS (2011) Development and validation of RP-HPLC method for quantitative estimation of vinpocetine in pure and Pharmaceutical Dosage Forms. Chromatogr Res Int 2011:1–7. https://doi.org/10.4061/2011/801656
21. Ghode PD, Pachauri AD, Sayare AS, Gaware A, Chaudhari R, Khandelwal KR, Ghode SP (2023) Development and validation of stability-indicating HPTLC method for the estimation of drotaverine hydrochloride in pharmaceutical dosage form. Int J Pharm Sci Res 14:2501. https://doi.org/10.13040/IJPSR.0975-8232.14(5).2501-10



Analytical Chemistry Letters

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tacl20

Development of RP-HPLC based analytical method for determination of cariprazine hydrochloride in bulk drug and pharmaceutical dosage form using box-behnken statistical design

Pallavi Chiprikar & Vinayak Mastiholimath

To cite this article: Pallavi Chiprikar & Vinayak Mastiholimath (2023) Development of RP-HPLC based analytical method for determination of cariprazine hydrochloride in bulk drug and pharmaceutical dosage form using box-behnken statistical design, Analytical Chemistry Letters, 13:3, 297-312, DOI: 10.1080/22297928.2023.2242857

To link to this article: https://doi.org/10.1080/22297928.2023.2242857

Analytical Chemistry Letters
https://www.tandfonline.com/journals/tacl
DOI: 10.1080/22297928.2023.2242857

Taylor & Francis
Taylor & Francis Group

ISSN: 2229-7928 (Print); ISSN: 2230-7532 (Online)

Research Article

Development of RP-HPLC based analytical method for determination of cariprazine hydrochloride in bulk drug and pharmaceutical dosage form using box-behnken statistical design

Pallavi Chiprikar and Vinayak Mastiholimath*

Department of Pharmaceutical Quality Assurance, KLE College of Pharmacy, Belagavi, Karnataka, India

Corresponding Author
Vinayak Mastiholimath
mastiholimath@rediffmail.com

Received 20 April 2023
Revised 22 July 2023
Accepted 22 July 2023

Abstract
A robust as well as precise technique aimed at determining the concentration of Cariprazine in bulk drugs along with formulations using RP-HPLC has been developed. The robustness study was optimized using Box Behnken statistical design. Separation was achieved using an Agilent 1260 Infinity II HPLC with an Agilent Zorbax Bonus RP column, as well as the mobile phase contained of methanol and 0.1% trifluoroacetic acid buffer (52.5:47.5) at a flow rate of 1 ml/min. The wavelength was set at 248 nm, and the method had an overall running period of 8 minutes, with the Cariprazine peak retention time at 3.83 min. The method exhibited linearity and accuracy for concentrations ranging from 80 to 120 µg/ml, with an R^2 of 0.999 and accuracy RSD of 0.2%, 0.13%, and 0.05% for 80%, 100%, and 120%, respectively. Stress stability testing under basic conditions revealed 53.32% degradation. The technique's limit of detection (LOD) and limit of quantification (LOQ) were very low, at 44.80 ng/ml and 135.60 ng/ml, correspondingly. These findings suggest that this method may be helpful during regular quality control evaluation of Cariprazine Hydrochloride in a variety of pharmaceutical formulations.

Keywords
Cariprazine Hydrochloride, RP-HPLC, Validation, Schizophrenia, Method development

Introduction

Schizophrenia is a mental illness that profoundly affects an individual's thoughts, emotions, and behaviors. The symptoms of this condition can cause an individual to feel disconnected from reality, which can be distressing for them and their loved ones. Performing routine activities can be challenging for someone with schizophrenia, but various effective treatments are available. With proper treatment, many individuals with schizophrenia can successfully attend school, work, and live independently while maintaining meaningful relationships with others[1]. Schizophrenia is a relatively uncommon mental illness, affecting approximately 1 in 300 people globally, which translates to over 24 million individuals. The prevalence rate among adults is slightly higher, at 0.4%, or 1 in 222 individuals. Typically, symptoms start in the latter stages of adolescence or the beginning of adulthood, with men often experiencing symptoms at an earlier age than women[2].

Cariprazine, also known as Vraylar in the US and Reagila in Europe is an atypical antipsychotic drug primarily used to treat schizophrenia, bipolar mania, and bipolar depression. It was approved for the first time in the United States in September 2015, for the treatment of schizophrenia[3]. The drug works by acting as a partial agonist on both D3 and D2 receptors, with a higher affinity for the D3 receptor. Cariprazine also shows partial agonist action at serotonin 5-HT1A receptors[4]. Phase III trials conducted in early 2012 demonstrated its efficacy in treating

schizophrenia and mania akathisia, while a Phase II trial conducted later in 2015 showed positive results in treating bipolar disorder I depression[5,6]. Cariprazine may also be used in conjunction with other treatments for severe depressive illness. It is prescribed to treat manic or mixed episodes, schizophrenia, and bipolar I disorder[7].

The chemical name (IUPAC) of Cariprazine hydrochloride is N′-[trans-4-[2-[4-(2,3-dichlorophenyl)-1-piperazinyl]ethyl] cyclohexyl]-N, N-dimethyl-urea with molecular formula $C_{21}H_{32}C_{l2}N_4O{\cdot}HCl$ (Fig. 1)[8,9]. It is a crystalline, white to off-white solid. Several organic solvents, such as methanol and dimethyl sulfoxide in which cariprazine HCl is soluble. Cariprazine is a BCS class II antipsychotic drug that contains piperazine. It is always synthesized in salt forms, specifically HCl, mesylate, and phenyl sulfonate due to its low solubility[10]. Cariprazine can cause akathisia and sleeplessness as the most common side effects. Unlike other antipsychotics, it does not affect prolactin levels or enhance the QT interval on an electrocardiogram. Short-term clinical studies have shown additional pyramidal effects, including dizziness, nausea, vomiting, anxiety, and constipation[11]. Although the prevalence of movement abnormalities in patients treated with Cariprazine was described as "quite high" in one study, it was deemed not significantly different from that reported in patients treated with placebo in another study[12].

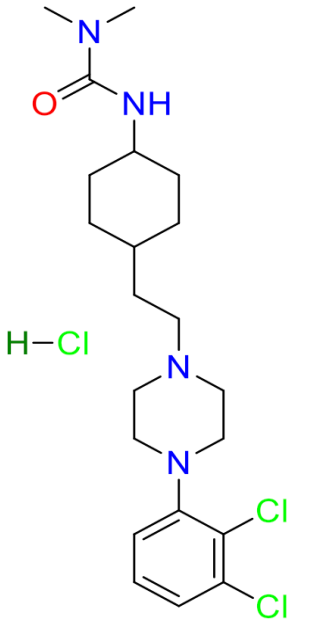


Figure 1. Chemical Structure of Cariprazine Hydrochloride

Analytical method development is an important stage during the development and manufacturing of pharmaceuticals, chemicals, and other products. It is the process of creating and optimizing a set of procedures or methods that are used to measure the physico-chemical characteristics of a constituent[13]. Accurate and reliable analytical methods are crucial for ensuring the quality, safety, and efficacy of products. Analytical methods are used to test for impurities, stability, and potency, among other factors. Analytical methods must comply with regulatory guidelines and standards set by agencies such as the FDA and EMA. The regulatory approval of a product depends on the development and validation of analytical techniques[14].

An extensive literature survey reveals that various attempts were made to develop an analytical technique aimed at the assessment of Cariprazine from bulk drugs and pharmaceutical dosage forms. Tiwari *et al.*[15] analysed cariprazine using a C-18 Inertsil ODS-3 column with a mobile phase consisting of a combination of 0.05 M ammonium acetate buffer and acetonitrile in a 50:50 v/v ratio. The pH was used to 4.8 through acetic acid and the analysis was performed at 217 nm with acetonitrile: water diluent combination in a 50:50 v/v ratio. The reported asymmetry value was 1.3, and the linearity R^2 value was 0.9954. However, the LOD and LOQ values were concluded to be 0.2 and 0.7 µg/mL, correspondingly, which are significantly higher than the industry standards. Additionally, the forced degradation data showed only 6.11% degradation in acid conditions, whereas identification of impurities through forced degradation requires at least three conditions showing degradation between 5-25%[15]. Ghumare *et al.*[16] utilized a chemsilods C-18 column (250 x 4.6 mm, 5 µm) with a mobile phase containing acetonitrile and ammonium acetate buffer (pH 4.8) in a 60:40 v/v ratio. The wavelength was set at 218 nm, and the flow rate was 1 mL/min

with a run time of 10 minutes. The linearity of the method was good with an R^2 value of 0.999. However, the theoretical plate was just barely within the ICH limits at 2956. The tailing factor was significantly high at 1.4, and it increased considerably with changes in the flow rate, resulting in a high relative standard deviation[16]. Pallekona *et al.*[17] developed a method for the analysis of Cariprazine and its degradant using a Waters C18 column (150 x 4.6 mm, 3.35-micron) with a flow rate of 1 mL/min and a mobile phase consisting of methanol and 0.1% orthophosphoric acid in a 50:50 (% v/v) ratio. The method had a short run time of only 6 minutes. The analysis was performed at a wavelength of 216 nm, and the reported theoretical plates were 4294 with a tailing factor of 1.20. The method was found to be linear with an R^2 value of 0.999. However, the standard cariprazine solution was stable for only 24 hours. The stress stability study revealed the deprivation of cariprazine in acidic, basic, peroxide, in addition to reduction circumstances, with degradation exceeding 23.5%[17].

In light of the limitations associated with current analytical methods for Cariprazine, such as low theoretical plate counts, high tailing factors, and the use of non-environmental friendly and costly solvents like acetonitrile, the study aimed to develop a stability-indicating RP-HPLC technique for determination of Cariprazine in both bulk drug and formulations. The main objective was to establish a precise and accurate method that can be utilized commercially in the industry for regular daily analysis work. To ensure that the method meets regulatory requirements, a Quality Target Profile (QTP) was set in accordance with ICH guidelines before development.

Material and methods

Chemicals and reagents

Cariprazine hydrochloride (Purity of Cariprazine reference standard 99.95%) was received as a gift sample from MSN Labs, Hyderabad. HPLC grade Methanol and Trifluoroacetic acid was obtained from Merck, India. In-house HPLC-grade water collected from the Milli-Q system was utilized in the experiments.

Instrumentation

The equipment used for development and validation was Agilent 1260 Infinity II with a DAD detector and quaternary pump. The software used was Openlab EzChrom Agilent software. Wet chemistry instruments were Aczet Analytical Balance and Labman Ultrasonicator, Vortex.

Preparation of 0.1% Trifluoroacetic acid

1 ml of Trifluoroacetic acid was transferred accurately in 1000 ml of type I HPLC water. The mixture was thoroughly stirred before being filtered through a 0.45 µm nylon filter.

Mobile phase mixture

The mobile phase was developed through the mixture of 475 mL of 0.1% Trifluoroacetic acid with 525 mL of methanol. Both solutions were mixed properly and passed with 0.45 µm nylon filter twice and sonicated for at 15 mins and degassed.

Preparation of diluent

The diluent was prepared by mixing equal volumes of Methanol and 0.1% Trifluoroacetic acid. The resulting solution was sonicated and filtered before use.

Preparation of cariprazine standard stock solution

5 mg of Cariprazine was weighed precisely and transmitted into 10 ml of a volumetric flask followed by dilution with diluent. The subsequent solution was vortexed and sonicated for 5 minutes to give a stock solution of 500 µg/ml. The solutions with concentrations between 80 and 120 µg/mL were obtained through additional dilutions. The calibration curve was plotted using these concentrations.

Preparation of cariprazine capsule formulation for assay

The weight of 10 capsules powder was weighed separately and the weight of each capsule was determined. As per the label claim, each capsule contained 1.5 mg of Cariprazine. The powder correspondent to 5 mg of Cariprazine was

weighed up accurately and transmitted in a 10 ml volumetric flask comprising 7 ml of diluent and mixed up properly. The volume was made up to 10 ml with diluent. The resulting solution was vortexed and sonicated for 10 minutes to give a stock solution of 500 µg/mL. The 10 mL volumetric flask was filled to the appropriate level with the pipette-out 1 ml stock solution. The mixture of the solution produced an ultimate concentration of 50 µg/mL.

Chromatographic conditions

The chromatographic separation was carried out by injecting (10 µL) sample into the HPLC system connected to Agilent Zorbax Bonus - RP (250 mm × 4.6 mm, 5 µm) operating at 1 mL/min flow rate, column temperature 30°C. A PDA detector with a 200-400 nm wavelength range was used to identify the analyte, and measurement was performed at 248 nm. The mobile phase included 0.1% trifluoroacetic acid and methanol.

Statistical tools

Design Expert® (Version 13; USA), was used to validate and optimize the analytical method. The quantitative analyses were completed using Microsoft Excel 2010 (Microsoft, USA).

Method development and validation

The ICH Q2 guidelines were used as a framework for developing and validating the technique of analysis[18]. The quality target profile (QTP) was decided before the development of the analytical method. The details are QTP are presented in (Table S1).

Specificity

Test is termed specific if the analyte can be evaluated without interference from any specific components like impurities, degradant, or excipients. It was validated by comparing the Cariprazine HCl chromatograms to a blank chromatogram.

System suitability

System's suitability was assessed in order to verify the resolution and repeatability. Six duplicates of the normal concentration (100 µg/mL) were performed (n=6). The ICH parameters for the system's applicability were calculated and the RSD of retention time, peak area, theoretical plates, and tailing factor were compared.

Linearity

Linearity study was performed by injecting samples of the concentration ranging from 80 to 120 µg/mL. When the peak areas from each standard were plotted versus concentration, the linearity formula and regression coefficient could be calculated. The samples were injected in triplicates and a chromatogram was recorded.

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

The capability of the technique to identify and measure the least amount of analyte is known as LOD and LOQ, correspondingly. The subsequent equations were used for determining the LOD and LOQ utilizing the standard deviation and slope of the regression line.

LOD = 3.3 Standard deviation (σ) / slope of the regression line (s)

LOQ = 10 Standard deviation (σ) / slope of the regression line (s)

Accuracy

Accuracy of the developed analytical method was evaluated by injecting the analyte at three different levels (80%, 100%, 120%) of concentration as per ICH guidelines. Three replicates of the samples were developed for each level. In order to determine recovery, the linearity equation was used.

Precision of the method (Repeatability)

Precision of the method was investigated through execution of six determinations of a similar sample.

Ruggedness

Ruggedness was carried out by two different analysts using the same HPLC system and different columns.

Robustness

Altering the settings, the robustness of the approach was investigated. The change in RSD was noticed after injecting the standard concentration three times. To determine its impact on peak retention and peak area, the robustness of the approach to changes in column temperature was assessed. The temperature varied at points including 2°C above and 2°C below fixed method temperature (30°C). The effect of changes in the mobile phase composition was evaluated by changing the composition. The robustness was evaluated by Response Surface Methodology (RSM) utilising Box-Behnken experimental design[19-21].

RSM analysis and optimization using Box-Behnken experimental design

Box-Behnken statistical design was used to develop and optimize the analytical method. Theoretical plates (Y1) and retention time (Y2) were regarded as dependent variables whereas changes in mobile phase composition (% Methanol) (A), column temperature (B), and flow rate (C) were regarded as independent variables. Table S2 provides a summary of the independent and dependent variable's coded levels. The actual optimization trials considering the Box-Behnken experimental design are presented in Table S3.

Solution stability

Solution stability was established through periodic investigation of the same sample solution.

Force degradation studies

Development of the HPLC technique's stability-indicating characteristic was performed in accordance with ICH requirements. Cariprazine hydrochloride was subjected to forced degradation experiments in acidic, alkaline, oxidative, UV, and dry heat environments.

Application of developed and validated analytical method

The developed and validated analytical technique was utilized towards checking its workability by determining entrapment efficiency and assay of the nanostructured lipid carrier and nasal spray respectively.

Preparation of nano-structured lipid carrier (NLC)

NLCs were formulated using the hot emulsification ultrasonication technique[22]. Concisely, quantified quantities of glyceryl monostearate (solid lipid, SL), Capmul MCM (liquid lipid, LL), Span 20 (amphiphilic emulsifier, 2% w/v), and Cariprazine were combined and heated to 70°C while being stirred to form uniform lipid phase. Tween 80 was then dissolved in distilled water and heated to 70°C. The hot aqueous phase was then added dropwise to the lipids, and the resulting dispersion was homogenized at 20,000 rpm for 3 minutes using an IKA Ultra-Turrax T18 homogenizer (IKA, Wilmington, NC, USA). A probe sonicator (Sonics Pvt. Ltd., India) was used to ultrasonicate the obtained pre-emulsion at 35% amplitude (10:10 on-off cycle). The prepared formulations were then refrigerated for further use in characterization.

% Entrapment efficiency

Variation within the original drug amount with the unbound or unentrapped amount of drug in the supernatant with regard to the total amount included in the nano lipid carrier formulation was used to evaluate the entrapment efficiency. The NLCs were centrifuged at 20000 rpm for 10 min to determine the degree of entrapment. Following centrifugation, 1 mL of the sample's supernatant layer was collected in a 10 mL volumetric flask. 5-6 mL of diluent was added, and the mixture was stirred for 2 mins before being built up to the required volume with diluent. This solution was pipetted out once more before being diluted to a volume of 10 mL using a diluent. The solution was injected, and the sample area was measured and used to assess how much drug was still present but not yet trapped[23].

$$Unentrapped\ Content\ (Uc) = \frac{Ru}{Rs}\ x\ Cs$$

where, Ru = Peak response of Cariprazine in the sample

Rs = Peak response of Cariprazine in the standard

Cs = Concentration of Cariprazine added in the NLCs (in mg/gm) &

$$\%\ Entrapment\ Efficiency\ (EE) = \left(\frac{(Td - Uc)}{Td}\right) \times 100$$

Where, Td = Total amount of drug added in NLC (in mg)

Uc = Amount of unentrapped drug (in mg)

In-house build nasal spray of cariprazine quantification (% Assay)

From the prepared sample of nasal spray, 1 ml of spray solution was accurately measured and transferred to a 10 ml volumetric flask, and 5-7 ml of diluent was mixed for 2 minutes and diluted and made up to the mark with diluent. (Concentration of Cariprazine = 100 µg/ml).% Assay was calculated using the following formula:

$$\%\ Assay = \frac{Ru}{Rs} \times \frac{Cs}{Cu} \times 100$$

where, Ru = Peak response of Cariprazine in the sample

Rs = Peak response of Cariprazine in the standard

Cs = Concentration of Cariprazine in standard (in µg/ml)

Cu = Concentration of Cariprazine in Sample (in µg/ml)

Results and discussion

Optimization of chromatographic condition

Numerous variations were made to various parameters throughout the course of the development of an HPLC technique for the measurement of cariprazine. The most significant change was made in the mobile phase ratio, which had a significant effect on the peak purity of Cariprazine. The optimal wavelength for the detection of Cariprazine was found to be 248 nm to minimize any interference of methanol in the chromatogram. Two different columns were evaluated during the development phase. The Agilent Zorbax SB-Aqs column showed the fronting and tailing of the peak, so it was replaced with the Agilent Zorbax Bonus RP column. The parameters such as Theoretical Plates (TP), Asymmetry, and peak purity were optimized according to the Quality Target Product Profile (QTP) criteria, and trial run 6 was found to have the best fit. Therefore, this run was used for validation.

The method development process is summarized in Table 1, which includes the different trials conducted and their respective results and observations. The chromatograms obtained during the development phase are shown in Fig. S1 - Fig S6. Fig. S7 shows the wavelength selection parameter for Cariprazine. Overall, the method development process was conducted according to established scientific principles, ensuring that the method is robust, accurate, and reliable. Table 2 summarises the optimised chromatographic conditions used during the validation of the method.

Specificity

Specificity is an essential parameter that is required to be evaluated as per regulatory guidelines such as ICH Q2(R1). The guidelines state that the method should be selective for the analyte of interest and not be interfered with by other components in the sample. Specificity

Table 1. Method development trials

Mobile phase and ratio	Wavelength	Retention time	Theoretical plates	Asymmetry	Peak purity
50 Methanol and 50 0.1% TFA	282	10.23	9313	1.22	0.93
60 Methanol and 40 0.1% TFA	215	4.83	8258	1.14	0.88
55 Methanol and 45 0.1% TFA	215	3.34	6274	1	1
52.5 Methanol and 47.5 0.1% TFA	215	3.47	7644	0.99	1
50 Methanol and 50 0.1% TFA	248	4.48	5840	1.02	1
52.5 Methanol and 47.5 0.1% TFA	248	3.85	6191	0.99	1

Table 2. Optimised chromatographic conditions

S. No.	Parameter	Condition
1	HPLC Instrument	Agilent 1260 Infinity II
2	Column	Agilent Zorbax Bonus RP (250 x 4.6 mm, 5 micron)
3	Wavelength	248 nm
4	Mobile Phase	52.5 Methanol: 47.5 of 0.1% Trifluoroacetic acid in water
5	Diluent	Methanol: 0.1% Trifluoroacetic acid in water (50:50)
6	Run time	8 minutes
7	Injection Volume	10 µl
8	Column Oven temperature	30°C
9	Flow Rate	1 ml/min

ensures that the method can detect and quantify the analyte of interest without interference from other components in the sample matrix[24]. Blank diluent and Cariprazine working standard were injected separately. From the chromatogram of the blank diluent, It was determined that there was not any disruption from blank peaks during the cariprazine's time of retention (Fig. S8). It was observed that the retention time of cariprazine was 3.85 minutes (Fig. S9).

System suitability

The system suitability results are tabulated in Table 3. The peak retention time was found to be 3.83 min. The number of theoretical plates in all working standard injections for cariprazine peak was not less than 2000, in addition, the tailing factor was found to be <2. The system's efficacy is indicated by greater theoretical plates and a reduced tailing factor. All of the parameters had an % RSD less than 2%.

Linearity

The calibration curve of cariprazine HCl concentration versus peak area was found to be linear (R^2=0.999, equation y = 17117x - 19735) within the concentration range of 80-120 µg/mL (Table 4). By plotting standard concentration *vs.* peak area, the calibration curve demonstrating the linearity of the developed technique was established (Fig. S10). The linearity overlay chromatogram of Cariprazine hydrochloride is presented in Fig. 2.

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

In HPLC technique development, LOD and LOQ are two important parameters that define the sensitivity of the technique. The LOD is well-defined as the lowermost amount of an analyte that can be consistently identified through a signal-to-noise ratio (S/N) of 3:1. This means that the signal from the analyte is at least 3 times greater than the noise level of the instrument. The LOD is a measure of the lowermost quantity of the analyte that can be distinguished, but not inevitably measured. On the other hand, the LOQ is defined as the lowermost quantity of an

Table 3. Results of system suitability studies

S. No.	Parameter	Mean	% RSD
1	Retention time (min)	3.83	0
2	Peak area	1695047	0.14
3	Theoretical plates	6156	1.05
4	Tailing factor	1.01	1.03

RSD Relative standard deviation, n=6

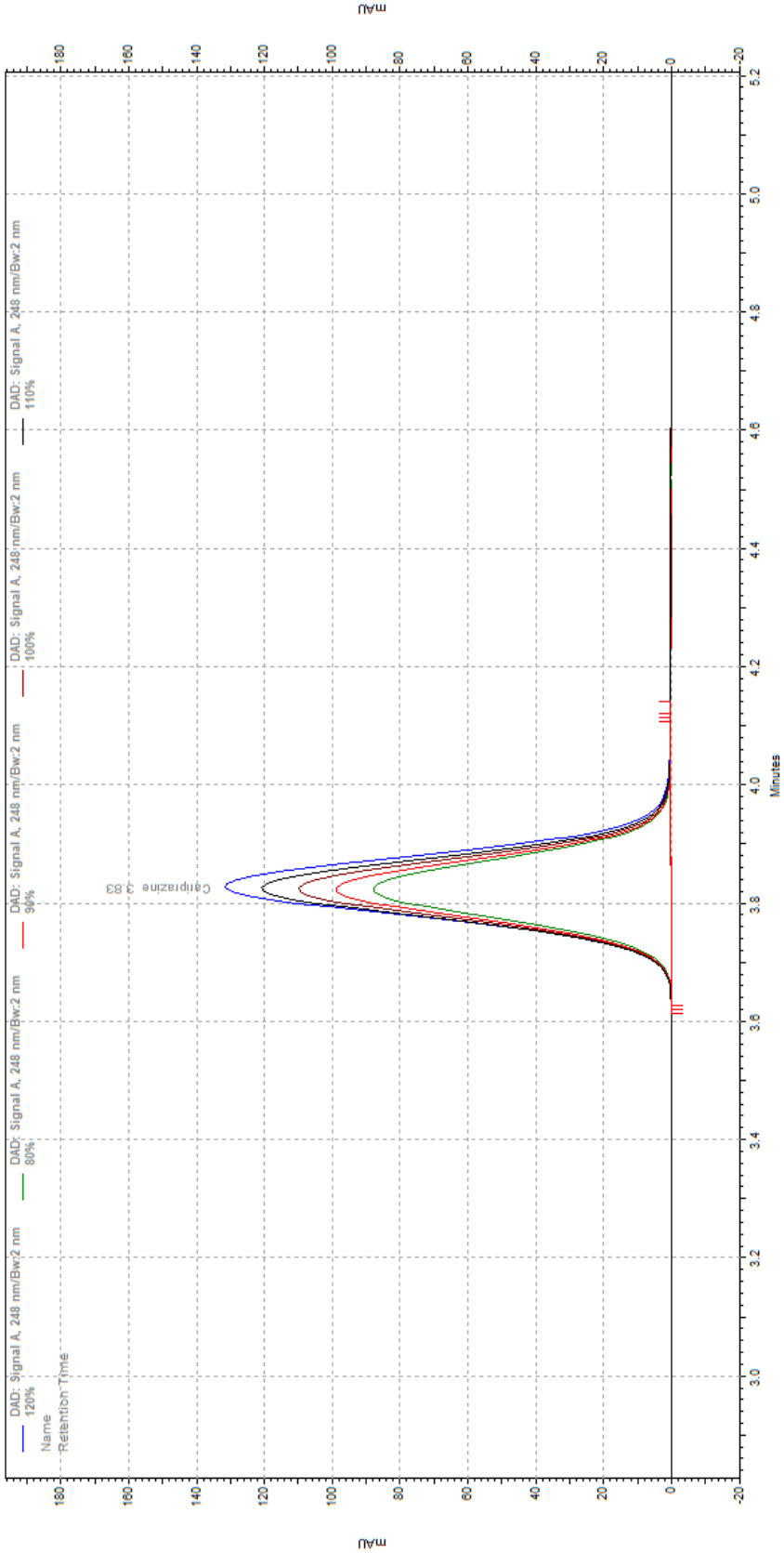


Figure 2. Linearity overlay chromatogram of cariprazine hydrochloride

Table 4. Linearity and regression analysis results

S. No.	Concentration (µg/mL)	Area
1	80	1353538
2	90	1512767
3	100	1692374
4	110	1871032
5	120	2030271
R^2		0.9995
Slope		17117
Y-intercept		19735

analyte that can be reliably quantified through a S/N of 10:1. This means that the signal from the analyte is at least 10 times greater than the noise level of the instrument. LOQ is a measure of the lowermost quantity of the analyte that can be accurately measured[23]. The LOD and LOQ of Cariprazine HCl were found to be 44.8 ng/mL and 135.6 ng/mL, correspondingly. The slope of the equation and the standard deviation were used in the current investigation to calculate LOD and LOQ.

Accuracy

Accuracy is a validation process to determine how closely the measured results of an HPLC method match the true values. The accuracy of an HPLC method is a measure of how well the method can provide results that are close to the true value of the sample being analyzed. Accuracy study is an essential step in the validation of an HPLC method, as it provides important information about the reliability and accuracy of the method for its intended use[16]. The recovery study was carried out to evaluate the precision of the suggested methodology. Standard proportions were spiked in three duplicates at three levels of the desired concentration (80, 100, 120%) and their drug content was examined. The % recovery was estimated by comparing the calculated drug concentrations to the nominal concentrations. According to the outcomes, the mean percentage of recovery for the three replicates at the levels of 80, 100, and 120% was 99.85%, 99.99%, and 99.86%, respectively. The results of the accuracy investigation are shown in Table 5.

Precision of method

Precision study is an essential part of HPLC method development. It helps towards ensuring the accuracy and reliability of the technique, identify sources of variation and error, and establish the limits of detection and quantification. By optimizing the precision of the HPLC technique, the sensitivity and range of the method can be improved, leading to more accurate and reliable analytical results[18]. The precision of the technique was assessed through factors like repeatability. In the precision study, six injections of standard solution were injected and % RSD was calculated. % RSD for the peak area is 0.14% for cariprazine HCl peak from 6

Table 5. Accuracy study results

% Level	Spiked Conc. (µg/ml)	Area	Amount Recovered (µg/ml)	% Recovery	RSD
	79.76	1353538	79.61	99.82	
80%	79.76	1351467	79.49	99.66	0.20
	79.76	1356876	79.81	100.06	
	99.70	1692374	99.54	99.84	
100%	99.70	1696751	99.80	100.10	0.13
	99.70	1695573	99.73	100.03	
	119.64	2030271	119.42	99.81	
120%	119.64	2031287	119.48	99.86	0.05
	119.64	2032144	119.53	99.91	

injections of working standard. The outcomes of the precision investigations are presented in Table 6.

Ruggedness

The Ruggedness study evaluates the robustness of the HPLC method by assessing its ability to produce consistent results when minor variations are introduced in the analytical conditions. These variations may include changes in equipment, analysts, reagents, or environmental conditions. The ruggedness study helps to identify the critical factors that distress the efficiency of the technique and their effect on the results. By evaluating the ruggedness of the method, it is possible to optimize the method's conditions and establish a range of acceptable variations[18]. This, in turn, improves the reliability as well as reproducibility of the technique. The Ruggedness study was carried out by two different analysts using the same HPLC system and different HPLC columns. The % RSD of 6 preparations was 0.10%. Table 7 summarizes results of precision, ruggedness and accuracy which indicates developed method was precise, accurate, and rugged.

Robustness

The robustness study evaluates the method's ability to remain unaffected by minor variations in the experimental circumstances, for example changes in pH, temperature, flow rate, and column aging. Parameters including mobile phase composition, and column temperature did not hinder the resolution. Because there were no apparent modifications in the chromatograms, it was shown that the RP-HPLC procedure was robust.

Table 6. Results of precision

S. No.	Area
1	1692374
2	1692474
3	1694578
4	1697853
5	1695472
6	1697528
Average	1695047
SD	2374.651
% RSD	0.14

Table 7. Results of precision, ruggedness and accuracy

Validation Parameter	% RSD	
Precision	0.14	
Accuracy	80 %	0.20
	100 %	0.13
	120 %	0.05
Ruggedness	0.10	

Optimization trials using Box-Behnken statistical design

The robustness of a chromatographic technique is defined as its resistance to minor changes resulting from variables like the mobile phase composition, flow rate, and column temperature. Using factors like the mobile phase composition and column temperature, Box-Behnken design was used in our investigation to determine the robustness of our technique. To assess the method's robustness, theoretical plates and retention time (min) were also taken into account as variables.

Box-Behnken statistical design was used to develop and optimize the analytical method for Cariprazine[19,20]. Mobile phase composition (A), alteration in column temperature (B), and alteration in flow rate (C) were deliberated independent variables whereas theoretical plates (Y1) and retention time (Y2) were considered dependent variables. The results of all optimization trials are presented in Table S4.

It was observed that in all trials, the theoretical plates were found to be more than 5000 while the retention time was also found to be less than 5 minutes. Theoretical plates and retention time are important parameters in analytical method development, particularly in chromatography. In analytical method development, the optimization of the number of theoretical plates and retention time is crucial for obtaining good resolution and accurate quantification of analytes. By increasing the number of theoretical plates, the resolution

of the separation can be improved. Adjusting the mobile phase composition and stationary phase properties can help optimize retention times for specific analytes. Therefore, a balance between theoretical plates and retention time is essential in analytical method development to achieve the desired separation and quantification of analytes[25]. The observed theoretical plates and retention time were found to be acceptable in all optimisation trials.

Statistical analysis of theoretical plates (Y1)

Theoretical plates refer to the theoretical number of equilibrium stages that a sample must pass through as it travels through a chromatographic column. The more theoretical plates a column has, the better its resolution or ability to separate different components of a mixture[26]. Theoretical plates are used to quantify the efficiency of a column. The polynomial equation for theoretical plates (Y1) can be presented below:

$$Y1 = +6148 + 62.75A + 43.87B + 16.62C + 57.50AB + 64AC \quad (1)$$

In the above equation, Y1 is theoretical plates, A is mobile phase composition (%), B is column temperature (°C) and C is the flow rate (ml/min). Overall the suggested quadratic model was found to be statistically nonsignificant by a *p*-value of 0.1097. The quadratic model was suggested for Y1 as shown in Table S5. The correlation coefficient (R^2) was found to be 0.9649 for Y1 representing a good fit quadratic model.

The model is indicated to be significant by the model F value of 9.17 (Table S5). The likelihood of noise producing an F-value this large is only 4.74%. Model terms that have *P*-values under 0.05 are considered significant. A, B, AB, and AC are important model terms in this situation. The 3D surface graph (Fig. 3) shows the impact of mobile phase composition (A), and variation in column temperature (B) on theoretical plates.

It was observed that the effect of mobile phase composition (A) and alteration in column temperature (B) on theoretical plates was found to be statistically substantial with a *P*-value less than 0.05 while flow rate (C) showed a non-significant outcome with a *P*-value greater than 0.05. A direct relation was observed between mobile phase composition, and alteration in column temperature, in addition to a number of theoretical plates as shown in Fig. 3.

Statistical analysis of retention time (Y2)

The retention time of a compound is influenced

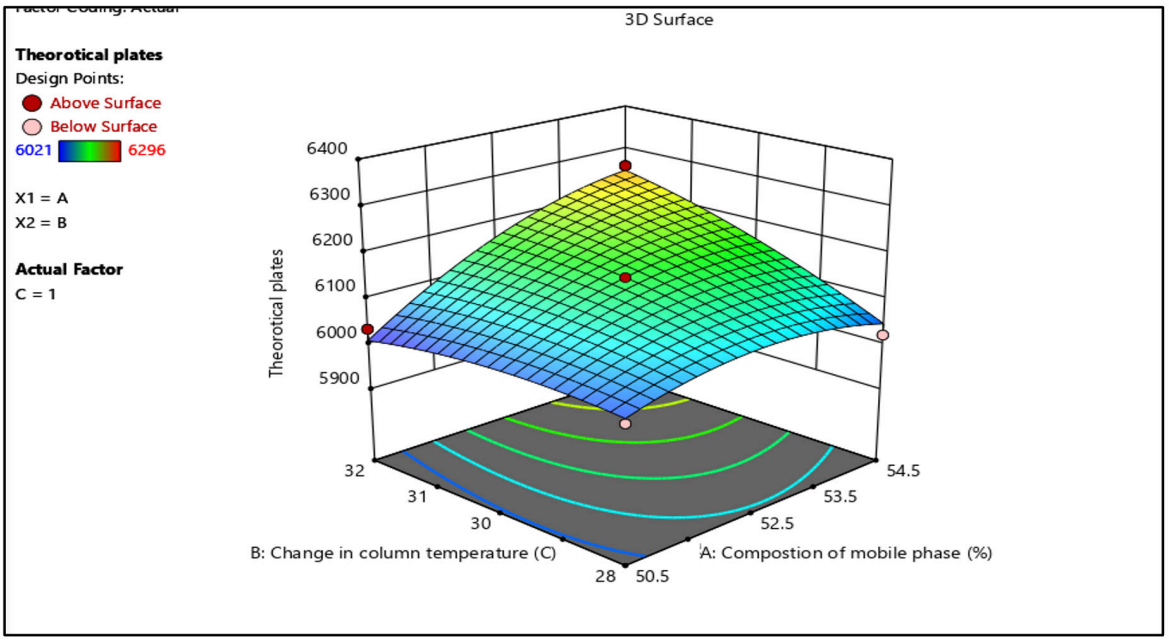


Figure 3. 3D surface responses of mobile phase composition (A) and alteration in column temperature (B) on theoretical plates

by numerous aspects for example the nature of the stationary phase, the mobile phase composition, the temperature, and the flow rate[27].

The polynomial equation for theoretical plates (Y2) can be presented below:

$$Y2 = +3.74 - 0.0638A + 0.0562B - 0.4975C \quad (2)$$

In the above equation, Y2 is retention time, A is mobile phase composition (%), B is column temperature (°C) and C is the flow rate (ml/min). With a *p*-value of 0.0001, the proposed linear model was discovered as being of statistical significance overall. For Y2, the linear model was proposed as shown in Table S6. For Y2, the correlation coefficient (R^2) of 0.9482 was discovered, indicating a well-fitting linear model. The model is indicated to be significant by the model F value of 54.93 (Table S6). The likelihood that noise would cause an F-value this large is less than 0.01%. Model terms that have *P*-values under 0.05 are considered significant. The effect of mobile phase composition (A) and change in column temperature (B) on retention time is presented in a 3D surface graph (Fig. 4). It was concluded that response Y1 and Y2 are robust and depends on factors mobile phase composition, column temperature and flow rate. Box-Behnken experimental design was employed successfully for evaluation of robustness. From results of ANOVA and analysis of response surfaces plots; it can be concluded that responses to therotical plates and retention time are robust for all the three factors mobile phase composition, flow rate and column temperature of the buffer within selected range.

Solution stability

HPLC is a broadly utilized system aimed at the analysis of various types of samples. The solution stability of the sample and the mobile phase is important in HPLC because it affects the accuracy, precision, and reliability of the results obtained. If the solution is unstable, the analyte may degrade, react with the mobile phase, or adsorb onto the column, leading to inconsistent results. Therefore, it is important to ensure that the sample and mobile phase are stable throughout the analysis. To ensure the solution stability in analytical method development using HPLC, it is important to carefully select the mobile phase and optimize the sampsle preparation and storage conditions. Additionally, regular monitoring of the solution stability throughout the analysis is crucial to ensure the accuracy and reliability of the results obtained[28,29]. The

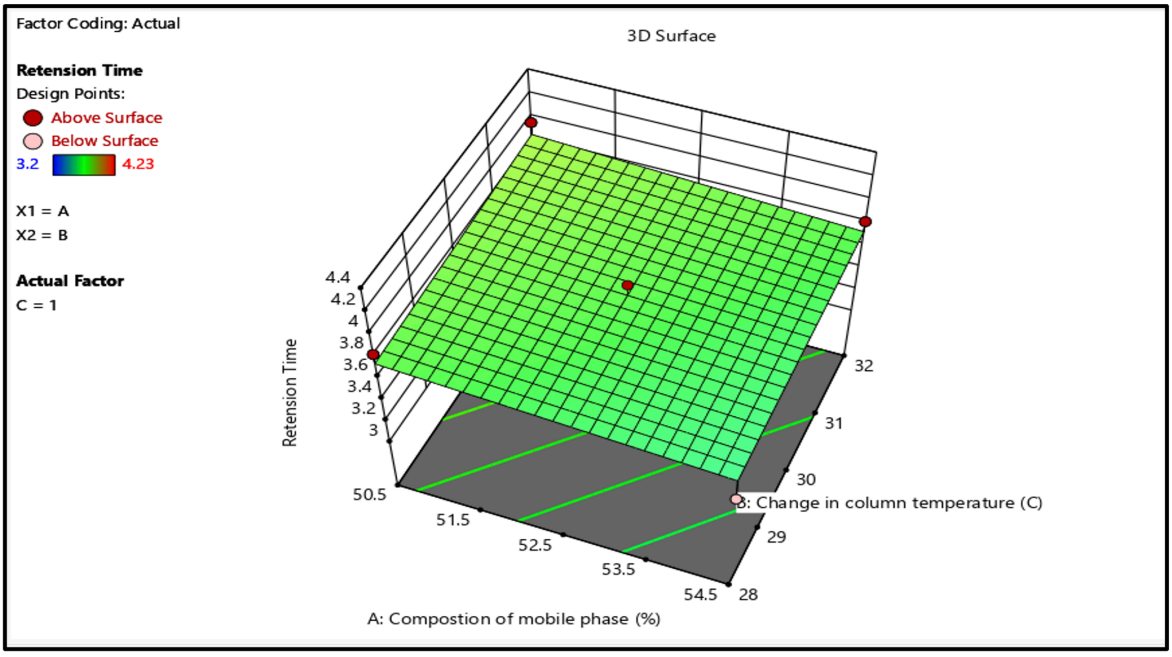


Figure 4. 3D surface responses of mobile phase composition (A) and change in column temperature (C) on retention time

stability of the Cariprazine HCl solution was checked by periodic analysis of the same sample solution and % RSD and cumulative % RSD of peak area was determined and found within the acceptable range. Table 8 shows the results for solution stability. It has been observed that the peak area did not significantly shift from the initial peak area even after 6 days. This clearly showed that the solution has excellent stability over a period of time.

Force degradation studies

The results of the force degradation study (acid, alkali, oxidation, UV, and heat) of cariprazine HCl are shown in Table 9. Cariprazine was found to degrade significantly in an alkaline environment. With only a 7.87% loss, it remained stable in the acidic environment. cariprazine HCl peak is well separated from any other peak due to diluents and degradation peak generated during stress conditions. Peak purity was observed outside of the acceptance criteria due to an interfering peak with the analyte peak. The interfering peak could be due to a placebo, as an acid, base, and heat. There was no interference due to diluent at RT of cariprazine HCl peak.

% Entrapment efficiency and assay

The developed and validated analytical technique of Cariprazine HCl was applied in the determination of % entrapment efficiency of NLC, assay of in-house nasal spray and marketed capsule formulation to check its workability. The average % encapsulation efficiency was found to be 85.61% while the assay of nasal spray was found to be 99.76% (Table 10). Based on

Table 8. Solution stability at room temperature

S. No.	Day	Area	% RSD	Cumulative % RSD
1	Day 0	1692374	-	-
2	Day 2	1691841	0.02	0.02
3	Day 4	1675476	0.5695	0.59
4	Day 6	1725877	1.2475	1.84

Table 9. Forced degradation study of Cariprazine HCl

S. No.	Sample name	Degradation condition	% Degradation
1	Acid	5N Hydrochloric acid 1 ml at 60ºC for 10 min	7.86
2	Base	1N Sodium Hydroxide 20 µl at RT for 10 min	53.32
3	Peroxide	30% Hydrogen peroxide 1 ml at 60ºC for 30 min	3.14
4	UV	254 nm for 24 hours	8.57
5	Dry heat	Thermal (80ºC for 3 hours in an oven)	3.31

Table 10. Summary of entrapment efficiency and assay results

S. No.	Sample ID	Area	Unentrapped Content	% Entrapment Efficiency
1	Standard	1696463	-	-
2	Sample 1	241067	1.421	85.79
3	Sample 2	247175	1.457	85.43
4	Sample 3	243951	1.438	85.62
Average			1.439	85.61
Assay (Nasal spray)			99.76%	
Assay (1.5 mg capsule brand name "Carilift" by Alkem Laboratories)			99.87%	

the validated method, assay was carried out on cariprazine 1.5 mg capsule brand name "Carilift" by Alkem Laboratories. The assay was found to be at 99.87% for the selected brand as shown in Table 10. The method was found to be workable and efficiently analyzed the in-house formulations.

Conclusion

The study has determined that the developed and validated HPLC technique is accurate, specific, reliable, and simple to use. Specificity testing revealed that no interferences were observed at the retention time of Cariprazine HCl, which was 3.8 minutes. All validation factors analyzed through the technique were inside suitable constraints. The method is also cost-effective due to the usage of fewer exclusive components in the mobile phase. The technique was effectively functional towards the quantitative determination of Cariprazine HCl in the forced degradation study as well as EE and an assay of in-house formulations. This study demonstrates the potential for future use of the method in regular quality control investigations of cariprazine HCl in different pharmaceutical formulations.

Declaration of conflict of interest

The authors declare that they have no competing financial interests.

Supplementary data

Table S1-S6 and Fig. S1-S10 are given as supplementary file.

References

1. **Lysaker, P.H., Pattison, M.L., Leonhardt, B.L., Phelps, S. and Vohs, J.L. (2018).** Insight in schizophrenia spectrum disorders: relationship with behavior, mood and perceived quality of life, underlying causes and emerging treatments. World Psychiatry. 17(1): 12-23.
2. **Ayano, G., Tesfaw, G. and Shumet, S. (2019).** The prevalence of schizophrenia and other psychotic disorders among homeless people: A systematic review and meta-analysis. BMC Psychiatry. 19(1): 1-14.
3. **Aringhieri, S., Carli, M., Kolachalam, S., Verdesca, V., Cini, E., Rossi, M., McCormick, P.J., Corsini, G.U., Maggio, R. and Scarselli, M. (2018).** Molecular targets of atypical antipsychotics: From mechanism of action to clinical differences. Pharmacology & Therapeutics. 192: 20-41.
4. **Calabrese, F., Tarazi, F.I., Racagni, G. and Riva, M.A. (2020).** The role of dopamine D3 receptors in the mechanism of action of cariprazine. CNS Spectrums, 25(3): 343-351.
5. **Durgam, S., Earley, W., Lipschitz, A., Guo, H., Laszlovszky, I., Németh, G., Vieta, E., Calabrese, J.R. and Yatham, L.N. (2016).** An 8-week randomized, double-blind, placebo-controlled evaluation of the safety and efficacy of cariprazine in patients with bipolar I depression. American Journal of Psychiatry. 173(3): 271-281.
6. **Gründer, G. (2010).** Cariprazine, an orally active D2/D3 receptor antagonist, for the potential treatment of schizophrenia, bipolar mania and depression. Current Opinion in Investigational Drugs. 11(7): 823-832. https://europepmc.org/article/med/20571978
7. **Batinic, B., Ristic, I., Zugic, M. and Baldwin, D.S. (2021).** Treatment of Symptom Clusters in Schizophrenia, Bipolar Disorder and Major Depressive Disorder With the Dopamine D3/D2 Preferring Partial Agonist Cariprazine. Frontiers in Psychiatry. 12: 784370.
8. **Chiprikar, P., Mastiholimath, V. and Balekundri, A. (2023).** HP-TLC Method Development of Cariprazine Hydrochloride for Applicative Quantification of Nanostructured Lipid Carriers. Chemistry Africa. 1-10.
9. **Chiprikar, P., Mastiholimath, V. (2022).** Method development and validation of Cariprazine hydrochloride in bulk by UV spectrophotometric method. Indian Journal of Novel Drug Delivery. 14(1): 52-57.
10. **Bharate, S.S. (2021).** Recent developments in pharmaceutical salts: FDA approvals from 2015 to 2019. Drug Discovery Today. 26(2): 384-398.
11. **Campbell, R.H., Diduch, M., Gardner,**

K.N. and Thomas, C. (2017). Review of cariprazine in management of psychiatric illness. Mental Health Clinician. 7(5): 221-229.

12. **Veselinović, T., Paulzen, M. and Gründer, G. (2014).** Cariprazine, a new, orally active dopamine D2/3 receptor partial agonist for the treatment of schizophrenia, bipolar mania and depression. Expert Review of Neurotherapeutics. 13(11): 1141-1159.
13. **Kolimi, P., Shankar, V.K., Shettar, A., Rangappa, S., Repka, M.A. and Murthy, S.N. (2022).** Development and Validation of HPLC Method for Efinaconazole: Application to Human Nail Permeation Studies. AAPS PharmSciTech. 23(1): 1-7.
14. **Chanda, A., Daly, A.M., Foley, D.A., Lapack, M.A., Mukherjee, S., Orr, J.D., Reid, G.L., Thompson, D.R. and Ward, H.W. (2015).** Industry perspectives on process analytical technology: Tools and applications in API development. Organic Process Research and Development. 19(1): 63-83.
15. **Tiwari, P., Hamrapurkar, P.D. (2020).** Development and validation of a stability indicating RP-HPLC method for determination of cariprazine in bulk drug. World Journal of Pharmaceutical Research. 84(4): 741-753.
16. **Ghumare, V.M., Lahu, H., Mhaske, M., Dhonde, P. (2022).** Development and Validation of Analytical method for Estimation of Cariprazine Hydrochloride in Bulk and Tablet Dosage Form by Using RP-HPLC Method. International Journal of Pharmaceutical Research and Applications. 7: 2280.
17. **Pallekona, S and Pawar, A.K. (2022).** Identification, separation and mass spectral characterization of degradants in Cariprazine HCl by LC-MS/MS/QTOF. Journal-of-Chemical-Metrology. 1307-6183.
18. **International Conference on Harmonisation of Technical Requirements For Registration Of Pharmaceuticals For Human Use.** ICH Harmonised Tripartite Guideline Validation Of Analytical Procedures: Text And Methodology Q2(R1).
19. **Muniz, V.M., Júnior, J.V.C., Aragão, C.F.S., deSouza, F.S. and Sampaio, F.C. (2023).** A HPLC method for simultaneous quantification of chlorhexidine and thymol using Box-Behnken design for robustness of the method assessment. Journal of Liquid Chromatography & Related Technologies. 1-12.
20. **Kumar, G., Mullick, P., Nandakumar, K., Mutalik, S. and Rao, C.M. (2022).** Box-Behnken Design-Based Development and Validation of a Reverse-Phase HPLC Analytical Method for the Estimation of Paclitaxel in Cationic Liposomes. Chromatographia. 85(7): 629-642.
21. **Beg, S., Kohli, K., Swain, S. and Hasnain, M.S. (2012).** Development and validation of RP-HPLC method for quantitation of amoxicillin trihydrate in bulk and pharmaceutical formulations using box-behnken experimental design. Journal of Liquid Chromatography and Related Technologies. 35(3): 393-406.
22. **Dadhania, P., Vuddanda, P.R., Jain, A., Velaga, S. and Singh, S. (2015).** Intranasal delivery of asenapine loaded nanostructured lipid carriers: formulation, characterization, pharmacokinetic and behavioural assessment. RSC Advances. 6(3): 2032-2045.
23. **Sivadasu, P., Gowda, D.V., Subramani, N.K., Vishweshwaraiah, B.M., Shivanna, S. and Hatna, S. (2020).** Direct Brain Targeted Nanostructured Lipid Carriers for Sustained Release of Schizophrenic Drug: Formulation, Characterization and Pharmacokinetic Studies. Indian Journal of Pharmaceutical Education and Research. 54(1): 73-84.
24. **Lavanya, G., Sunil, M., Eswarudu, M.M., Eswaraiah, M.C., Harisudha, K. and Naga Spandana, B. (2013).** Analytical Method Validation: An Updated Review. International Journal of Pharmaceutical Sciences and Research, 4(4): 1280-1286.
25. **Ito, M., Shimizu, K. and Nakatani, K. (2021).** Three approaches to improving performance of liquid chromatography

using contour maps with pressure, time, and number of theoretical plates. Journal of Chromatography A. 1637: 461778.

26. **Kumar, L., Sreenivasa Reddy, M., Managuli, R.S. and Pai, K.G. (2015).** Full factorial design for optimization, development and validation of HPLC method to determine valsartan in nano-particles. Saudi Pharmaceutical Journal. 23(5): 549-555.
27. **Sun, L., Jin, H.Y., Tian, R.T., Wang, M.J., Liu, L.N., Ye, L.P., Zuo, T.T. and Ma, S.C., (2017).** A simple method for HPLC retention time prediction: Linear calibration using two reference substances. Chinese Medicine. 12(1): 1-12.
28. **Saadati, N., Abdullah, M.P., Zakaria, Z., Sany, S.B.T., Rezayi, M. and Hassonizadeh, H. (2013).** Limit of detection and limit of quantification development procedures for organochlorine pesticides analysis in water and sediment matrices. Chemistry Central Journal. 7(1): 1-10.
29. **Shankar, V.K., Wang, M., Ajjarapu, S., Kolimi, P., Avula, B., Murthy, R., Khan, I. and Murthy, S.N. (2022).** Analysis of docosanol using GC/MS: Method development, validation, and application to ex vivo human skin permeation studies. Journal of Pharmaceutical Analysis. 12(2): 287-292.

INDIAN PHARMACY GRADUATES ASSOCIATION (IPGA)
MP. STATE BRANCH

Organizing International Conference *(Hybrid Mode)* on 06th May 2023

In collaboration with
Dr. A.P.J. Abdul Kalam University
Indore

"...Nurturing Talents To Success"

Co-Host
Indore Institute of Pharmacy
Indore

Indore Institute of Pharmacy

CERTIFICATE OF PARTICIPATION

This is to certify that Dr/Mr/Mrs/Ms. **PALLAVI CHIPRIKAR** of **DEPARTMENT OF PHARMACEUTICAL QUALITY ASSURANCE, KLE COLLEGE OF PHARMACY, BELAGAVI, KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH (KAHER) BELAGAVI, KARNATAKA, INDIA.** has actively participated in International Conference on Challenges and Opportunities in Future Pharmaceutical Research and Innovations (COFPRI 2023) held on 06th May 2023 at University Cultural Centre DAVV, Takshila Campus, Indore.

He/She also presented paper/delivered Invited talk/Judge/chaired a Technical Session/ Organizing Committee Member/ titled **HP- TLC METHOD DEVELOPMENT OF CARIPRAZINE HYDROCHLORIDE FOR APPLICATIVE QUANTIFICATION OF NANOSTRUCTURED LIPID CARRIERS**

Dr. Karunakar Shukla
President IPGA MP, Convener

Dr. Dinesh Mishra
Secretary IPGA MP, Convener

Dr. Revathi A Gupta
Treasurer IPGA MP, Convener

Dr. Rakesh Kumar Jatav
Vice President IPGA MP, Co-Convener

Dr. Rakesh Patel
Co-Convener

JSS ACADEMY OF HIGHER EDUCATION & RESEARCH
(DEEMED TO BE UNIVERSITY) MYSURU

JSS Academy of Higher Education & Research

JSS College of Pharmacy, Mysuru

National Conference on

Pharmaceutical Sciences

2022

CERTIFICATE

OF PRESENTATION

This is to certify that

Dr./ Mr./ Ms. PALLAVI CHIPRIKAR

has participated as a **Delegate** and presented a **poster** entitled "Novel RP-HPLC Method Development And Validation for Cariprazine HCL & It's Forced Degradation Study" in the National Conference on **"Pharmaceutical Sciences 2022"** held on 8th and 9th April 2022 organized by Department of Pharmaceutics at JSS College of Pharmacy, Mysuru.

Dr. D.V.Gowda
Professor & Head,
Department of Pharmaceutics
JSS College of Pharmacy,
Mysuru

Dr. T M Pramod Kumar
Dean, Faculty of Pharmacy &
Principal, JSS College of Pharmacy,
Mysurul

NPTEL Online Certification

(Funded by the MoE, Govt. of India)

Elite

This certificate is awarded to

PALLAVI RAOSAHEB CHIPRIKAR

for successfully completing the course

Health Research Fundamentals

(NIE-ICMR e-Certificate course: NIeCer 101)

with a score of **76** %

Online Assignments	23.54/25	Proctored Exam	52.5/75

Total number of candidates certified in this course: **622**

Dr. Manoj Murhekar
Scientist 'G' & Director
ICMR-National Institute of Epidemiology, Chennai

Prof. Balram Bhargava
Secretary to Govt. of India, Dept. of Health Research
& Director-General, Indian Council of Medical Research

Prof. Andrew Thangaraj
NPTEL, Coordinator
IIT Madras

ICMR INDIAN COUNCIL OF MEDICAL RESEARCH | NIE NATIONAL INSTITUTE OF EPIDEMIOLOGY

Jul-Sep 2022

(8 week course)

FREE ONLINE EDUCATION swayam शिक्षित भारत, उन्नत भारत

Roll No: NPTEL22HS73S23430152

To validate the certificate

No. of credits recommended: 2 or 3

विज्ञान एवं प्रौद्योगिकी विभाग
DEPARTMENT OF
SCIENCE & TECHNOLOGY

Azadi Ka Amrit Mahotsav

Certificate of Participation

This certificate is awarded to

Pallavi Chiprikar

For successful participation in a hands-on-training program on
Analytical and Bioanalytical Techniques for Pharmaceuticals,
held under the initiative,
Synergistic Training Program Utilizing the Scientific and
Technological Infrastructure (STUTI)
Department of Science and Technology-India,
October 31 - November 6, 2022.

Dr. Ratnesh Jain DST-STUTI ICT Coordinator	**Prof. P. R. Vavia** Director ICT Bhubaneswar, Chief Guest	**Prof. S.C. Si** Dean and Program Coordinator