DEVELOPMENT AND EVALUATION OF MODIFIED RELEASE TABLETS OF LOVASTATIN AND OLEANOLIC ACID

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

in

Pharmaceutics

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2026

DECLARATION

I, hereby declared that the presented work in the thesis entitled **"Development and Evaluation of Modified Release Tablets of Lovastatin and Oleanolic acid"** in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision of **Dr. Narendra Kumar Pandey**, working as Professor, in the School of Pharmaceutical Sciences of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

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CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled **"Development and Evaluation of Modified Release Tablets of Lovastatin and Oleanolic acid"** submitted in fulfillment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.)** in the School of Pharmaceutical Sciences, is a research work carried out by Mandave Satish Vasant, 41800902, is bonafide record of his original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

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Name of supervisor: Dr. Narendra Kumar Pandey

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ABSTRACT

Cardiovascular diseases (CVDs) remain one of the leading causes of morbidity and mortality worldwide, with hyperlipidemia recognized as a major risk factor. Conventional pharmacological approaches often necessitate multiple medications targeting different aspects of lipid metabolism, such as cholesterol synthesis and absorption. However, these multi-drug regimens frequently lead to poor patient adherence. To address this challenge, this research aimed to develop and evaluate modified-release tablet formulations incorporating both lovastatin and oleanolic acid using two distinct delivery approaches: bilayer tablets and core and coat tablets.

The formulations were designed to provide an immediate release of lovastatin, ensuring rapid inhibition of HMG-CoA reductase, which is crucial for reducing endogenous cholesterol synthesis. Concurrently, oleanolic acid was incorporated in a sustained-release manner to inhibit intestinal Acyl-CoA:cholesterol acyltransferase (ACAT), thereby reducing dietary cholesterol absorption over an extended period. This dual-mechanism approach was intended to enhance therapeutic efficacy while improving patient compliance.

A comprehensive methodology was employed to achieve optimal formulation. Preformulation studies were conducted to evaluate the physicochemical properties of the active pharmaceutical ingredients (APIs) and excipients. Drug compatibility was confirmed using Fourier Transform Infrared (FTIR) spectroscopy, Differential Scanning Calorimetry (DSC), and X-ray Diffraction (XRD) analysis, ensuring the stability of the formulation components. A robust, validated Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed for simultaneous quantification of lovastatin and oleanolic acid.

Optimization of the formulations was conducted using a 3^2 full factorial design to determine the ideal concentrations of excipients for achieving the desired drug release profiles. The optimized bilayer tablet demonstrated 95.23% release of lovastatin within 30 minutes, ensuring rapid therapeutic action. Meanwhile, oleanolic acid exhibited a controlled release of 97.13% over 12 hours in the core and coat tablet, providing sustained cholesterol absorption inhibition. Mathematical modeling of the dissolution profiles indicated zero-order kinetics for oleanolic acid, signifying a

concentration-independent release, whereas lovastatin followed the Korsmeyer-Peppas model, indicative of a diffusion-controlled mechanism.

Stability studies conducted under accelerated conditions over six months demonstrated that both formulations retained their physicochemical integrity, confirming their robustness for potential clinical application. Pharmacokinetic evaluations in New Zealand white rabbits further validated the formulations, with lovastatin reaching peak plasma concentration (Cmax) within 0.5 hours and oleanolic acid attaining its Cmax at 6 hours, ensuring sustained therapeutic levels over 48 hours.

The efficacy of the developed formulations was assessed in hyperlipidemic rabbit models. Both the bilayer and core and coat tablets significantly improved lipid profiles, reducing total cholesterol, triglycerides, and LDL-cholesterol while enhancing HDL-cholesterol levels. Notably, the core and coat tablet formulation demonstrated superior efficacy compared to the bilayer tablet and lovastatin monotherapy, indicating its potential as a more effective treatment option.

These findings highlight the promise of dual-mechanism, modified-release formulations for improved lipid management. By combining immediate inhibition of cholesterol synthesis with sustained inhibition of cholesterol absorption, these formulations offer an innovative therapeutic strategy that enhances patient compliance and efficacy. This research supports the potential clinical application of such formulations in the management of hyperlipidemia and associated cardiovascular risks.

Keywords: Lovastatin, Oleanolic acid, modified release tablets, Bilayer tablets, Core and coat tablets, Hyperlipidemia,

ACKNOWLDGEMENT

The journey of a PhD is never walked alone. It is a path shaped by the support, guidance, and love of many. As I reach the culmination of this journey, I am filled with immense gratitude.

I wish to express my deepest appreciation to my supervisor, **Dr. Narendra Kumar Pandey**, Professor, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, whose invaluable guidance, thoughtful insights, and unwavering encouragement have been the cornerstone of this research. His mentorship has not only shaped this thesis but has also inspired my growth as a researcher and individual.

I am profoundly thankful to my organization, **Shri Vithal Education and Research Institute**, for providing an intellectually stimulating environment and all necessary resources. The institute has continuously fostered a culture of academic excellence and innovation, which has greatly enriched my research experience. I am especially grateful to **Hon. Prof. Dr. B. P. Ronge**, Secretary of the Institute, for his steady support and encouragement, which have been instrumental throughout this journey. His visionary leadership and genuine concern for the growth of researchers like me have been a source of constant motivation and inspiration. I feel privileged to be part of such a forward-thinking and supportive academic community.

I would also like to extend my heartfelt thanks to all the **staff members of SVERI's College of Pharmacy (Poly.), Pandharpur**, for their constant encouragement, cooperation, and understanding. Your support throughout this endeavor has meant a great deal to me.

To my dear colleagues and friends, **Mr. Shardul Nalbilwar**, **Mr. Amit Ligade**, **Mr. Hemant Bansode**, **Dr. Sachin Jadhav and Dr. Rachana Bhimanwar**, thank you for your continued encouragement, insightful discussions, and invaluable friendship. I would also like to extend my sincere thanks to **Dr. Santosh Gandhi,** Professor, AISSMS College of Pharmacy, Pune for providing essential research

facilities and valuable support during the course of my work. Support of you all has helped me stay motivated and focused throughout this long and demanding journey.

To my beloved parents, **Mrs. Satyabhama Mandave** and **Late Mr. Vasant Mandave**, whose blessings, love, sacrifices, and unwavering belief in me have always been my anchor. Everything I am, and everything I strive to be, is rooted in your values and support.

To my beloved wife, **Mrs. Manisha Mandave**, your unwavering love, boundless patience, and quiet strength have been the pillars on which I have leaned throughout this journey. In moments of doubt and weariness, your belief in me never wavered. To our precious daughters, **Swara Mandave** and **Ishwari Mandave**, your joyful laughter, curious hearts, and innocent smiles have been my guiding light on even the darkest days. You three are the soul of my world, and this achievement is as much yours as it is mine.

This thesis is dedicated to all of you for your love, belief, and presence in my life. Without you, this journey would have never been possible.

Satish Vasant Mandave

DEDICATION

I dedicate this thesis with profound love, gratitude, and respect to my beloved father, **Late Mr. Vasant M. Mandave**.

Your unwavering support, values, sacrifices, and encouragement have always been the guiding force in my life. Although you are no longer physically present with us, your blessings, teachings, and memories continue to inspire me every day and give me the strength to achieve my goals.

This achievement is a humble tribute to your dreams, hard work, and the principles you instilled in me. Your love and guidance remain forever in my heart and will continue to illuminate my path throughout my life.

I sincerely offer this work in your cherished memory.

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LIST OF ABBREVIATIONS:

Symbol/ Abbreviations	Full form
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
ACAT	Acyl-CoA cholesterol acyltransferase
CVDs	Cardiovascular diseases
CHD	Coronary heart disease
LDL	Low density lipoprotein
VLDL	Very low density lipoprotein
HDL	High density lipoprotein
TG	Triglycerides
TC	Total cholesterol
LV	Lovastatin
OA	Oleanolic acid
CYP3A4	Cytochrome P450 3A4
HPMC K100	Hydroxypropyl methylcellulose k100
MCC	Microcrystalline cellulose
MCTs	Medium-chain triglycerides
CAD	Coronary artery disease
PAD	Peripheral artery disease
DDS	Drug delivery system
IBD	Inflammatory bowel disease
3D	Three-dimensional
IR	Immediate release
SR	Sustained release
EC	Ethyl cellulose
GIT	Gastrointestinal tract
CAP	Cellulose acetate phthalate
FTIR	Fourier transform infrared
DSC	Differential scanning calorimetry
XRD	X-ray Diffraction
LOD	Limit of Detection
LOQ	Limit of Quantitation
TD	Tapped density
HR	Hausner ratio
CI’s	Carr’s index
BD	Bulk density
SLS	Sodium lauryl sulfate
DT	Disintegration test
SSG	Sodium starch glycolate
IS	Internal standard
BN	Benorilate
LC-MS/MS	Liquid chromatography - tandem mass spectrometry
MRM	Multiple reaction monitoring
V	Volt
eV	Electronvolt

Symbol/ Abbreviations	Full form
v/v	Volume/volume
OPA	Orthophosphoric acid
RT	Retention time
SD	Standard deviation
RSD	Relative standard deviation
sec	Second
min	Minute
T_{max}	Time to peak drug concentration
C_{max}	Maximum concentration
$t_{1/2}$	Half-life
AUC	Area under the curve
K_{el}	Elimination constant
mL	Milliliter
mg	Milligram
%	Percentage
rpm	Rotations per minute
℃	Degree centigrade
h	Hour
ACN	Acetonitrile
cm	Centimeter
cm^2	Centimeter square
cm^{-1}	Centimeter inverse
et al.	And co-workers
Fig.	Figure
ICH	International Conference on Harmonization
RH	Relative humidity
RP- HPLC	Reserved Phase High Performance Liquid Chormatography
µL	Microliter
µg	Microgram
ng	Nanogram
Conc.	Concentration
Temp.	Temperature

1. INTRODUCTION

1.1 Background and Significance

Lovastatin, a statin, and oleanolic acid, a triterpenoid, are two bioactives with cholesterol-lowering property. The bilayer and core and coat tablet approach for their co-administration is a new technique presenting various benefits including better drug efficacy, fewer side effects, and increased patient adherence. Lovastatin works by inhibiting the HMG-CoA reductase enzyme, thus decreasing blood cholesterol level (Frishman and Rapier, 1989). To maintain the cholesterol levels in the body it is also necessary to regulate the cholesterol which comes from the diet. The enzyme Acyl-CoA:cholesterol acyltransferase (ACAT), found within intestinal cells, plays a essential part in cholesterol absorption in the small intestine by converting cholesterol into cholesteryl esters (Helgerud et al., 1981). The deposition of cholesteryl esters in macrophages results in the formation of foam cells, a key characteristic of the initial stages of atherosclerosis. Oleanolic acid reduces cholesterol level by inhibiting the activity of Acyl-CoA: cholesterol acyltransferase hence reduces the risk of atherosclerosis (Luo et al., 2024). In order to get dual actions i.e. control of cholesterol by liver synthesis (Lovastatin) and from absorption from diet (Oleanolic Acid) it is ideal to prepare bi layer tablets or core and coat tablet by using combination of two drugs. From this combination one drug is instant release and one drug is sustained release.

Although current pharmacological strategies effectively reduce cholesterol levels, most therapies focus on single mechanistic pathways and often require long-term high-dose administration, which may increase the risk of adverse effects and poor patient adherence. Existing oral formulations do not adequately address simultaneous control of hepatic cholesterol synthesis and intestinal absorption within a single, optimized dosage form. This limitation highlights the need for advanced drug delivery strategies capable of integrating multi-targeted therapy with controlled release characteristics.

1.1.1 Overview of Cardiovascular Diseases (CVDs)

Cardiovascular diseases (CVDs) confer a major global health burden, with conditions like hypertension as well as hypercholesterolemia being essential in their pathogenesis. The combination of these risk factors has been shown to have a harmful

synergistic impact on vascular health, ultimately leading to the development of coronary heart disease (CHD) (Soppert et al., 2020). The management of CVDs typically requires the concomitant administration of antihypertensive and hypo-lipidemic agents, underscoring the necessity for individualized treatment strategies. Novel strides in pharmaceutical technology, for instance the invention of bilayer gastro retentive tablets, are promising better therapeutic outcomes for CVD patients. Using direct compression technology, these bilayer tablets can offer distinctive release profiles of antihypertensive and anti-lipidemic agents, thereby optimizing drug delivery tailored to the specific requirements of this patient cohort (Mourya et al., 2023). Furthermore, extrusion-based 3D printing has shown promise in fabricating complex oral solid dosage forms, like multi-active tablets for ailments for example hypertension combined with diabetes. These pioneering techniques might transform personalized medical delivery, eventually augmenting patient adherence and treatment success in the context of cardiovascular health (Peng et al., 2024).

1.1.2 Importance of managing hyperlipidemia

Hyperlipidemia is a condition characterized by elevated lipid levels in the bloodstream, presents a considerable risk element for cardiovascular ailments, counting MI's and CHD's. The management of hyperlipidemia is vital in diminishing these hazards and enhancing patient prognoses. The combinational use of lipid-lowering medications, for example Simvastatin, Lovastatin, and Ezetimibe, has exhibited potential in effectively decreasing cholesterol levels. This technique assists not only in decreasing the intestinal cholesterol transport to the liver but also provides a tolerable option for lipid control in hyperlipidemic patients (McPherson et al., 2024). Adhering to a structured pharmacotherapy approach, as advised by the National Cholesterol Education Program (NCEP), can help reduce and potentially reverse coronary atherosclerosis, especially in those with coronary heart disease. Through adherence to these holistic treatment techniqueologies, medical professionals can markedly influence the prevention and control of hyperlipidemia, consequently enhancing the cardiovascular health of patients (Kuller, 2006).

1.1.3 Current therapeutic strategies for hyperlipidemia

Furthermore, concerning contemporary therapeutic protocols for hyperlipidemia, statins have established a primary role in treatment owing to their potent capability in

diminishing LDL cholesterol levels. Lovastatin, as a constituent of the statin category, has seen extensive application attributable to its proficiency in mitigating cardiovascular risk (Khatiwada and Hong, 2024). Besides, adjunctive therapies, notably oleanolic acid, have exhibited potential in augmenting lipid profiles. Oleanolic acid, a triterpenoid entity sourced from assorted plants, has manifested lipid-decreasing attributes (Wang et al., 2022). When conjoined with lovastatin in bilayer tablet compositions, these two agents potentially operate synergistically to deliver heightened therapeutic outcomes for hyperlipidemia management. By means of an exhaustive analysis of the pharmacological actions and clinical results of lovastatin and oleanolic acid, this innovative treatment options proffer a noteworthy approach to tackling hyperlipidemia.

1.2 Lovastatin: Pharmacology and Clinical Use

Lovastatin, belonging to the statin meds category, influences its pharmacological impact by restraining the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, essential in the cholesterol biosynthesis process. Restraining this enzyme, lovastatin eventually lowers the cholesterol levels in the blood, specifically low-density lipoprotein (LDL) cholesterol. This function has made lovastatin integral to managing hyperlipidemia and averting cardiovascular conditions. Clinical trials have indicated that lovastatin not just diminishes cholesterol levels proficiently, but also lowers the risk of cardiovascular incidents like myocardial infarctions and strokes. Besides, lovastatin has indicated anti-inflammatory and antioxidant traits that might add to its cardiovascular advantages beyond cholesterol-decreasing impacts (Morofuji et al., 2022). Combining lovastatin with other elements, like oleanolic acid, in bilayer and core and coat tablets might boost its therapeutic effects and widen its clinical application in the future. More scientific inquiry is necessary to uncover lovastatin’s full capabilities in different disease contexts and to perfect its medical application.

1.2.1 Mechanism of action (MOA) of lovastatin

Because of functioning as a competitive inhibitor of HMG-CoA reductase, lovastatin efficaciously lessens cholesterol synthesis within the liver. By impeding this chief enzyme in the mevalonate pathway, lovastatin ultimately fallouts in a decrease in the formation of cholesterol precursors in addition to, consequently, devaluation in total

cholesterol amounts in the body. Lovastatin's MOA primarily revolves around its capacity to obstruct the transformation of HMG-CoA to mevalonate, an essential step in cholesterol synthesis. These inhibition outcomes in an augmented expression of LDL receptors on hepatocyte surfaces, enhancing the removal of LDL cholesterol from the bloodstream. Moreover, lovastatin shows anti-inflammatory and antioxidant characteristics that contribute to its overall cardiovascular protective effects (Seenivasan et al., 2008). Additionally, the synergistic interactions of lovastatin and oleanolic acid, as evidenced in bilayer tablets, suggest an innovative technique to boosting the pharmacological advantages of these substances for the management of hypercholesterolemia and related cardiovascular conditions.

1.2.2 Therapeutic benefits and limitations

Notwithstanding the potential therapeutic advantages of bilayer core and coat tablets comprising both Lovastatin and Oleanolic Acid, a variety of limitations necessitate addressing. A concern is the prospect of drug interactions between Lovastatin and Oleanolic Acid, possibly resulting in adverse effects or diminished efficacy (Neuvonen et al., 2006). Moreover, the bioavailability of the two drugs could diverge when co-formulated in a bilayer tablet, influencing the overall therapeutic outcomes. Additionally, the production process of bilayer tablets tends to be intricate and expensive, rendering it less accessible for widespread application in contrast to traditional tablets. Nonetheless, with further investigation and refinement, these limitations can be surmounted to enhance the therapeutic virtues of bilayer tablets and Core and Coat tablet of Lovastatin and Oleanolic Acid in treating conditions for example hypercholesterolemia and inflammation. Overall, combining Lovastatin and Oleanolic Acid in bilayer tablets and modified release tablet offers a distinctive opportunity to amplify therapeutic outcomes via synergistic effects. Employing both drugs within a singular dosage form may curtail the necessity for multiple medications and intricate dosing schedules, thereby improving patient adherence and convenience (Gajanan et al., 2022). Furthermore, the controlled release characteristics of bilayer and core and coat tablets can aid in maintaining stable plasma concentrations of the drugs, promoting more consistent therapeutic effects over time (Adepu and Ramakrishna, 2021). Despite these benefits, it is essential to meticulously evaluate the pharmacokinetics and pharmacodynamics of each drug to optimize

bilayer tablet formulations, ensuring safety and efficacy in clinical application (Kim et al., 2025).

1.2.3 Challenges in bioavailability and first-pass metabolism

Although innovative oral drug delivery systems such as bilayer and core-and-coat tablets do not completely bypass hepatic first-pass metabolism, they can significantly mitigate its impact by optimizing drug release, absorption timing, and residence in the gastrointestinal tract. Conventional immediate-release formulations of lovastatin are associated with rapid dissolution and extensive pre-systemic metabolism, leading to low and variable oral bioavailability. Similarly, oleanolic acid suffers from poor aqueous solubility and limited intestinal permeability, further restricting its systemic availability. (Zhou and Zhou, 2015).

Modified release approaches, including bilayer and core-and-coat tablets, can improve effective bioavailability by prolonging drug release, maintaining drug concentrations within the optimal absorption window, and reducing peak-related metabolic loss. By modulating release kinetics and minimizing premature degradation in the gastrointestinal environment, these systems enhance the fraction of drug available for absorption, thereby improving therapeutic consistency rather than directly circumventing first-pass metabolism. Such formulation-based optimization represents a rational strategy to address bioavailability limitations inherent to conventional oral therapies (Jiang et al., 2016).(Azman et al., 2022). Furthermore, integrating excipients that either inhibit metabolic enzymes or boost absorption can significantly augment the bioavailability of these substances (Patel et al., 2020). Addressing the issues of bioavailability and first-pass metabolism, bilayer and coat and core tablets present a viable approach to enhance the therapeutic effectiveness of combining lovastatin and oleanolic acid in treatment regimens.

1.3 Oleanolic Acid: Pharmacological Profile

The pharmacological characteristics of oleanolic acid, as a principal active component in the bilayer and core and coat tablets consisting of Lovastatin and Oleanolic Acid, hold a significant impact in managing cholesterol level. Investigations have pinpointed oleanolic acid as an essential active compound in the traditional Chinese medicine Ganweikang (GWK) tablet (Xu et al., 2023). Oleanolic acid, in conjunction with compounds for example ferulic acid and ursolic acid, targets essential proteins

including NFKB1, AKT1, and STAT1 (Kang et al., 2021). Furthermore, the combination therapy strategy highlighted in guidelines stresses the importance of addressing the comprehensive lipid profile, like triglycerides and HDL levels, to reduce cardiovascular risks. This extensive strategy underlines the potential of merging oleanolic acid with statins to attain optimal therapeutic outcomes for both dyslipidemias, presenting a promising path for future pharmaceutical interventions (Jie, 1995).

Despite extensive evidence supporting the lipid-lowering and anti-atherosclerotic properties of oleanolic acid, its clinical translation remains constrained by poor oral bioavailability and limited formulation-focused investigations. Most studies emphasize pharmacological activity without addressing delivery-related challenges or its integration with established lipid-lowering agents such as statins. This gap underscores the necessity of formulation-driven approaches to fully exploit the therapeutic potential of oleanolic acid in combination therapy.

1.3.1 Source and chemical structure of oleanolic acid

Oleanolic acid, being a triterpenoid compound of frequent occurrence in botanical entities for example olive trees, garlic, and assorted medicinal herbs, establishes itself as particularly noteworthy (Rodriguez-Rodriguez, 2015). Its chemical architecture includes a pentacyclic core featuring a hydroxyl grouping at the C-3 position, categorizing it as a hydroxy triterpenoid. Considerable attention has been directed towards this compound because of its extensive range of pharmacological properties encompassing anti-inflammatory, anti-cancer, and hepatoprotective activities (Sen, 2020). The biosynthesis of oleanolic acid proceeds via the mevalonic acid pathway, initiating from squalene undergoing epoxidation and subsequent cyclization to actualize the core triterpenoid skeleton (Szakiel et al., 2003). Subsequent molecular manipulations for example hydroxylation and glycosylation, at diverse positions on the molecule, can lead to the development of various analogs that may possess augmented bioactivity (Luchnikova et al., 2020). Acquiring an understanding of oleanolic acid's source and chemical structure is vital for delving into its therapeutic capabilities and crafting new drug delivery systems, for example bilayer and coat and core tablets that amalgamate oleanolic acid with other bioactives like lovastatin.

1.3.2 Pharmacological activities

Furthermore, the pharmacological aspects of lovastatin and oleanolic acid have been considerably examined. Lovastatin is identified for its anti-inflammatory traits, which might aid in lessening inflammation in several ailments like atherosclerosis and arthritis (Koushki et al., 2021). Moreover, lovastatin has been discovered to possess anti-cancer attributes, whilst oleanolic acid has anti-lipidemic characteristics. The amalgamation of these pharmacological functionalities in bilayer and coat and core tablets might propose a promising technique for addressing intricate diseases, delivering multiple advantages in a solitary dosage form. Prospective investigations should concentrate on elucidating the foundational mechanisms of these effects and enhancing the formulation for improved therapeutic potency.

1.4 Combination Therapy: Rationale and Benefits

Considering the intricacies of diseases coupled with drug resistance development, there is an observable surge in attention towards combination therapy as a viable strategy to augment treatment results. The amalgamation of two or more pharmaceutical agents with distinct mechanisms of action enables the addressing of multiple pathways implicated in the disease's progression, thereby potentially enhancing efficacy while mitigating adverse effects. Specifically referencing bilayer and modified release tablets composed of Lovastatin and Oleanolic Acid, the justification rests in their respective and complementary impacts on lipid metabolism and antilipidemic mechanisms (Ansarullah et al., 2009). Lovastatin, classified as a statin, predominantly inhibits cholesterol biosynthesis, whereas oleanolic acid demonstrates anti-cholesterol activity. The integration of these compounds in a unified dosage can engender synergistic outcomes, which may translate to superior therapeutic efficacy and improved patient compliance (Lehar et al., 2009). Thus, the merits of combination therapy, particularly in this scenario, underscore the critical need to investigate innovative drug delivery systems and formulation techniqueologies.

While combination therapy is widely recognized for its potential to improve therapeutic outcomes, existing research predominantly focuses on co-administration rather than rational formulation design. Limited attention has been given to developing delivery systems that synchronize distinct release profiles with the

pharmacokinetic and pharmacodynamic requirements of individual drugs. Consequently, there remains a critical need for dosage forms, such as bilayer or core-and-coat tablets, that can strategically modulate drug release to maximize synergistic efficacy.

1.4.1 Potential advantages in treating hyperlipidemia and CVDs

Further, the administration of bilayer and core and coat tablets that comprise lovastatin and oleanolic acid manifests potential in hyperlipidemia treatment alongside the prevention of cardiovascular diseases (CVDs). Lovastatin: a recognized statin drug is efficacious in lowering LDL cholesterol levels. The formulation's inclusion of oleanolic acid, a natural triterpenoid exhibiting anti-cholesterol benefits (García-González et al., 2023; Tobert, 1988). Oleanolic acid contributes to the reduction of inflammation and oxidative stress critical elements in the onset and worsening of CVDs (Vasarri et al., 2025). The combination of lovastatin with oleanolic acid may improve treatment outcomes for hyperlipidemic patients prone to CVDs. Research validating these benefits is essential to corroborate the efficacy of this innovative treatment technique and demonstrate its potential impacts on patient care and results. Continued clinical trials could examine the therapeutic advantages of this combination therapy, opening a new path for hyperlipidemia management and CVDs prevention.

1.4.2 Research Gap and Study Rationale

Hyperlipidemia remains a major risk factor for cardiovascular diseases, necessitating effective and sustained therapeutic interventions. Among the available pharmacological agents, lovastatin a widely used statin acts by inhibiting HMG-CoA reductase, thereby reducing hepatic cholesterol synthesis. Similarly, oleanolic acid, a natural triterpenoid, has demonstrated promising lipid-lowering activity, primarily through modulation of intestinal cholesterol absorption and other metabolic pathways. Although both agents exhibit significant individual efficacy, their combined therapeutic potential has not been fully explored**.**

Most of the existing literature focuses on the individual pharmacological effects of statins or natural compounds, with limited attention given to their integration into a single dosage form**.** This represents a critical gap, as combination therapy could potentially offer synergistic benefits by targeting multiple pathways involved in lipid

metabolism. Furthermore, there is a lack of systematic research on advanced drug delivery systems**,** such as bilayer or core-and-coat tablets, that can effectively deliver such combinations with controlled and site-specific release profiles**.**

Another important limitation of current therapeutic approaches lies in pharmacokinetic challenges, including poor aqueous solubility, low oral bioavailability, and extensive first-pass metabolism, particularly in the case of lovastatin. In addition, conventional formulations fail to achieve temporal synchronization of drug release**,** which is essential for optimizing the dual mechanisms of action of lovastatin and oleanolic acid. The absence of polymer-based modified release systems that can address these issues further highlights the unmet need in this area.

In this context, the development of a bilayer or core-and-coat tablet system presents a promising strategy. Such a system can be designed to provide immediate release of lovastatin for rapid inhibition of hepatic cholesterol synthesis, along with sustained release of oleanolic acid for prolonged modulation of intestinal lipid absorption. This approach not only improves the pharmacokinetic profile of both drugs but also enhances their therapeutic synergy.

Therefore, the present study is aimed at the formulation and evaluation of a novel bilayer/core-and-coat tablet combining lovastatin and oleanolic acid. The proposed system seeks to overcome the limitations of conventional dosage forms by improving bioavailability, ensuring controlled drug release, and enhancing overall therapeutic efficacy. Ultimately, this strategy is expected to provide a more effective, safe, and patient-compliant option for the long-term management of hyperlipidemia and associated cardiovascular disorders.

1.5 Modified Release Drug Delivery Systems (DDS)

The selection of appropriate polymers constitutes a pivotal aspect in the design of modified release DDS. Polymers possess a significant function in modulating the release rate of medicinal substances from the dosage medium (Karolewicz, 2016). Specifically, in bilayer tablets which encompass lovastatin and oleanolic acid, thoughtful attention must be directed towards polymer selection to achieve optimal drug release patterns (Akhtar et al., 2020). The compatibility between the polymer and the medicinal agent, alongside the swelling and erosion properties of the polymer,

will exert a direct influence on the release kinetics of the drugs (Kavanagh and Corrigan, 2004). Moreover, the mechanical attributes of the polymer must be considered to ensure the tablets sustain their integrity throughout storage and transportation phases. Through meticulous polymer selection for bilayer and coat and core tablets, researchers can customize drug release profiles to fulfill specific patient requisites and enhance therapeutic outcomes.

2. LITERATURE REVIEW

2.1 Drug Review

2.1.1 Lovastatin

Lovastatin is used in the direction of lowering elevated total and LDL cholesterol levels whereas increasing HDL cholesterol, making it effective in managing hypercholesterolemia and mixed dyslipidemia. It helps decrease the threat of CVD's, together with heart attacks and strokes, by slowing the progression of atherosclerosis. Additionally, it is prescribed for individuals with familial hypercholesterolemia and can aid in managing high triglyceride levels. By inhibiting HMG-CoA reductase, Lovastatin decreases cholesterol synthesis, promoting overall heart health and preventing complications associated with high cholesterol (Zolkiflee et al., 2017).

IUPAC Name: (1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2-methylbutanoate.

Proprietary names: Mevacor, Altocor, Altoprev.

Molecular formula: $C_{24}H_{36}O_5$

Molecular weight: 404.55 g/mol

Structural formula:

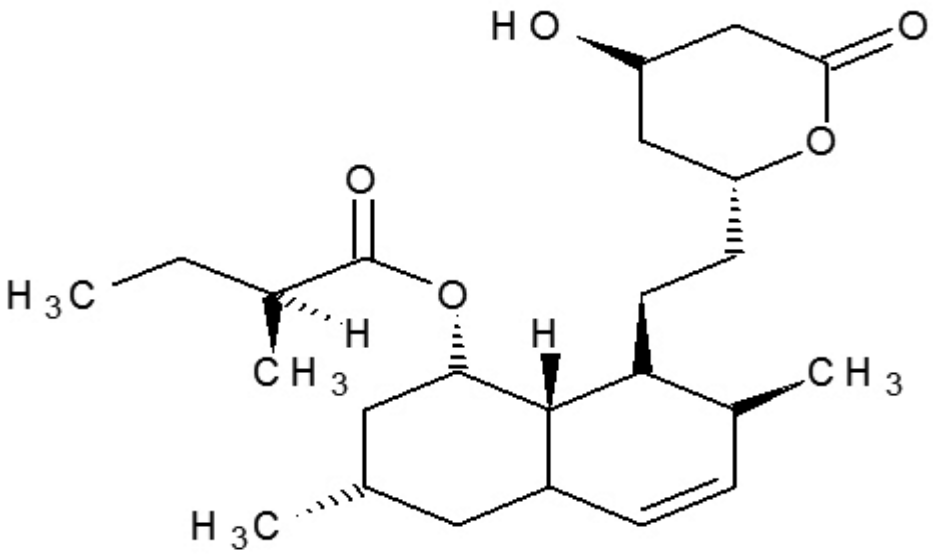

Fig. 1: Chemical Structure of Lovastatin

Solubility: Practically insoluble in water; freely soluble in chloroform, methanol, and ethanol.

Category: Lipid-lowering agent (Statins).

Melting point: 174-178°C

Volume of distribution: 30 L

Protein binding: >95%

Biological half-life: 3 hours

Bioavailability: Less than 5% (because of first-pass metabolism).

Description: Lovastatin exists as a white, non-hygroscopic crystalline powder.

Storage: Store tightly closed, away from light and moisture.

MOA: Lovastatin is a prodrug that is hydrolyzed to its active form, which hinders HMG-CoA reductase. This enzyme is liable for converting HMG-CoA into mevalonate, a cholesterol precursor. The inhibition of HMG-CoA reductase results in reduced cholesterol production and increased expression of LDL receptors, which enhances the clearance of LDL from the bloodstream.

Pharmacokinetics: Lovastatin is extensively metabolized in the liver. Peak plasma concentration occurs within 2-4 hours. It undertakes widespread first-pass metabolism via CYP3A4 enzymes. Excretion occurs via bile (83%) and urine (10%).

Adverse Effects: Common side effects: headache, nausea, dizziness, myopathy, elevated liver enzymes. Rarely, rhabdomyolysis and hepatotoxicity can occur.

Drug Interactions: Strong CYP3A4 inhibitors for example ketoconazole, itraconazole, ritonavir increase lovastatin levels. Grapefruit juice should be avoided as it can enhance its plasma concentration.

Uses: Lovastatin is indicated for hypercholesterolemia, primary prevention of cardiovascular diseases, and mixed dyslipidemia (Goswami et al., 2013).

Dosage Forms: Mevacor, Altoprev

Several studies have explored modified-release formulations of lovastatin to overcome its low oral bioavailability and extensive first-pass metabolism. Approaches such as matrix tablets, sustained-release systems, and polymer-based formulations have demonstrated improved dissolution behavior and prolonged drug release, resulting in enhanced therapeutic consistency. However, most reported formulations focus on single-drug delivery and do not address combination strategies or layer-

specific release optimization, highlighting the need for advanced systems such as bilayer and core-and-coat tablets.

2.1.2 Oleanolic Acid

Oleanolic acid, a naturally occurring pentacyclic triterpenoid, has garnered significant attention for its cardioprotective effects. Found abundantly in plants for example olives (Olea europaea), fruits, and medicinal herbs, oleanolic acid exerts a range of biological activities that directly benefit cardiovascular health. Its chemical structure, characterized by a triterpenoid backbone, enables interactions with diverse cellular targets, making it a promising therapeutic. Oleanolic acid has been shown to modulate lipid metabolism, decreasing TC, TG, and LDL-C levels while increasing high-density lipoprotein cholesterol (HDL-C). These lipid- lowering effects are mediated through the regulation of key enzymes convoluted in lipid biosynthesis and absorption, for example Intestinal acyl-CoA: cholesterolacyl transferase (ACAT) and peroxisome proliferator-activated receptor-alpha (PPAR-α) (Nie et al., 2015). By improving lipid profiles, oleanolic acid contributes to reduced plaque burden and enhanced cardiovascular protection (Pollier and Goossens, 2012).

Oleanolic acid has been widely reported for its antihyperlipidemic, hepatoprotective, and antioxidant properties. Preclinical studies indicate that oleanolic acid modulates lipid metabolism by regulating cholesterol biosynthesis and enhancing lipid clearance pathways. Despite its promising therapeutic potential, its clinical translation remains limited due to poor aqueous solubility and low oral bioavailability, necessitating formulation-based strategies to improve its delivery and efficacy in hyperlipidemia management.

Physicochemical properties

Oleanolic acid is a naturally occurring pentacyclic triterpenoid with the molecular formula $C_{30}H_{48}O_3$ and a molecular weight of about 456.7 g/mol. It typically appears as a white to off-white crystalline powder and exhibits high thermal stability with a melting point above 300 °C. Due to its hydrophobic nature, oleanolic acid is practically insoluble in water but soluble in organic solvents such as ethanol, methanol, chloroform, and DMSO. Its low aqueous solubility and high lipophilicity (high log P) contribute to formulation challenges, especially for oral drug delivery.

IUPAC Name: (4aS,6aR,6aS,6bR,8aR,12aR,14bS)-4a,6a,6b,8a,11,11,14b-heptamethyl-2-oxo-2,3,4,5,6,6a,6b,7,8,9,10,12,12a,13,14,14b-hexadecahydropicene-4a-carboxylic acid.

Molecular Formula: $C_{30}H_{48}O_3$

Molecular Weight: 456.71 g/mol

Structural formula:

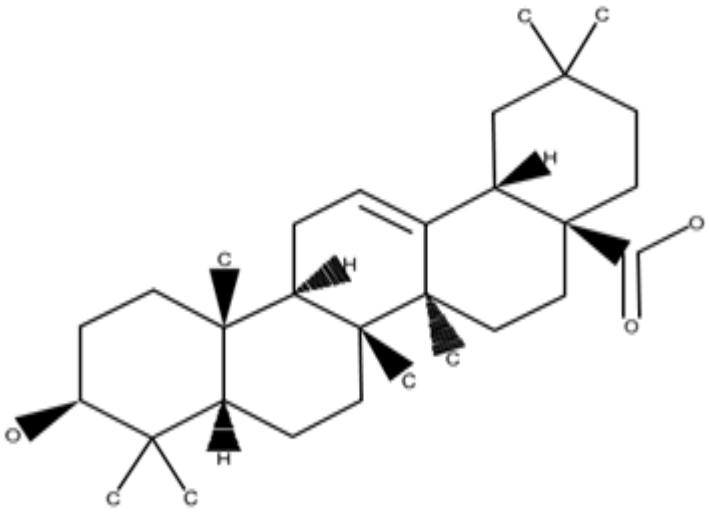

Fig. 2: Chemical Structure of Oleanolic acid

Category: It is a pentacyclic triterpene and is known for its various bioactive properties, including hepatoprotective, anti-inflammatory, and lipid lowering effects.

Solubility: Insoluble in water; soluble in ethanol, chloroform, and DMSO.

Pharmacokinetics

The pharmacokinetic profile of oleanolic acid has been studied primarily in animal models. In rats, oleanolic acid shows low permeability and limited absorption due to its physicochemical properties, leading to poor oral bioavailability. Intravenous studies in rats indicate dose-linear pharmacokinetics, with moderate clearance and a half-life in the range of 40–53 minutes after intravenous administration

MOA: Oleanolic acid helps protect the liver by decreasing oxidative stress and inflammation. It influences the NF-κB signaling pathway and also displays anti-diabetic, anti-hypertensive, and antimicrobial properties. (Lin et al., 2016).

2.2 Excipients Review

2.2.1 Hydroxypropyl Methylcellulose K100 (HPMC K100)

HPMC K100 is a semi-synthetic, water-soluble polymer used as a binder, film-former, and controlled-release agent in pharmaceutical formulations. It enhances drug solubility and stability while providing sustained-release properties in oral dosage forms. HPMC K100 is usually used in ophthalmic solutions, coatings for tablets, and as a thickener in suspensions. It is biocompatible, non-toxic, and widely utilized in both immediate and extended-release DDS. Its capability to form a gel upon hydration makes it an essential excipient in controlled drug release applications (Tamasree Majumder et al., 2016).

IUPAC Name: Hydroxypropyl methyl cellulose

Molecular Formula: $C_{56}H_{108}O_{30}$

Molecular Weight: Varies based on polymer length

Solubility: Soluble in water; insoluble in organic solvents

Category: Pharmaceutical binder, film-forming agent, controlled-release polymer

Description: A white to off-white, fibrous or granular powder used in pharmaceuticals as a viscosity-increasing agent, stabilizer, and film former.

Uses: Hydroxypropyl Methylcellulose (HPMC) K100 is a pharmaceutical and industrial-grade polymer used primarily as a binder, film-former, and controlled-release agent in oral drug formulations, especially in sustained-release tablets. It is also used as a thickener, emulsifier, and stabilizer in food, cosmetics, and ophthalmic products like artificial tears for dry eyes. Additionally, it functions as a coating agent in tablet manufacturing and improves drug solubility and bioavailability in pharmaceutical applications (Arefin et al., 2016; Khiste et al., 2021; Vijaya et al., 2015).

2.2.2 Ethyl Cellulose

Ethyl Cellulose is a water-insoluble polymer widely used as a coating agent, binder, and controlled-release matrix in pharmaceutical formulations. It provides a protective barrier in tablets and pellets, ensuring sustained drug release and stability. Because of its hydrophobic nature, it is usually employed in microencapsulation to modify drug release profiles. Ethyl Cellulose is also used in taste-masking applications and as a

thickener in topical formulations. Its versatility makes it a key excipient in both oral and transdermal drug delivery systems (Wasilewska and Winnicka, 2019).

IUPAC Name: Ethyl ether of cellulose

Molecular Formula: $C_{23}H_{34}O_{11}$

Molecular Weight: Varies based on polymer chain length

Solubility: It does not dissolve in water but is soluble in organic solvents for example ethanol and methanol.

Category: Film former, water-insoluble polymer

Description: A white to light tan powder used as a coating material for controlled-release formulations.

Uses: Ethyl cellulose is a versatile polymer used in pharmaceuticals, food, and industrial applications. In pharmaceuticals, it serves as a film-forming agent, coating material, and controlled-release excipient for tablets and capsules. In the food industry, it acts as a thickener, stabilizer, and emulsifier. Industrially, it is used in paints, varnishes, adhesives, and microencapsulation because of its water-insoluble and thermoplastic properties (Murtaza, 2015; Rao et al., 2012).

2.2.3 Sodium Starch Glycolate (SSG)

IUPAC Name: Sodium carboxymethyl starch

Molecular Formula: $C_2H_4O_3Na$ ($C_6H_{10}O_5$)

Molecular Weight: Variable

Solubility: It is mostly insoluble in organic solvents but expands when exposed to water.

Category: Superdisintegrant

Description: A white or off-white, doorless, tasteless powder that absorbs water rapidly, leading to tablet disintegration.

Uses: SSG is a superdisintegrant widely used in the pharmaceutical industry to enhance the dissolution and bioavailability of oral tablets and capsules. It helps tablets break down quickly in the GIT, allowing for faster drug absorption. SSG is usually used in formulations of immediate-release tablets, including pain relievers, antibiotics, and antihistamines. It is derived from starch (potato, maize, or rice) and is generally regarded as safe for pharmaceutical use (Aneela Manzoor, 2021; Putra et al., 2024; Shah and Augsburger, 2002).

2.2.4 Croscarmellose Sodium

IUPAC Name: Sodium salt of cross-linked carboxymethylcellulose

Molecular Formula: Variable $C_8H_{16}NaO_8$

Molecular Weight: Variable

Solubility: Swells in water, insoluble in organic solvents

Category: Superdisintegrant

Description: A white or off-white powder utilized towards improve the disintegration of tablets and capsules.

Uses: Croscarmellose Sodium is a superdisintegrant used in pharmaceutical tablets and capsules to enhance their dissolution and absorption. It helps tablets break apart quickly upon contact with water or gastric fluids, improving the bioavailability of active ingredients. It is usually used in oral medications, dietary supplements, and over-the-counter (OTC) drugs to ensure rapid and effective drug release (Monton et al., 2023; Parfati and Rani, 2018).

2.2.5 Magnesium Stearate

IUPAC Name: Magnesium octadecanoate

Molecular Formula: $C_{36}H_{70}MgO_4$

Molecular Weight: 591.27 g/mol

Solubility: Practically insoluble in water and alcohol

Category: Lubricant

Description: A fine, white powder with hydrophobic properties, usually used to prevent tablet sticking and capping.

Uses: Magnesium stearate is usually used as a lubricant, anti-adherent, and flow agent in the pharmaceutical and food industries. In medications and supplements, it prevents ingredients from sticking to manufacturing equipment, ensuring uniform tablet formation and smooth powder flow. It is also used in cosmetics, personal care products, and food processing as an emulsifier and stabilizer. Despite some concerns, it is generally recognized as safe (GRAS) when used in regulated amounts (Hobbs et al., 2017; Leinonen et al., 1992; Zarmpi et al., 2020).

2.2.6 Talc

IUPAC Name: Hydrated magnesium silicate

Molecular Formula: $Mg_3Si_4O_{10}(OH)_2$

Molecular Weight: 379.27 g/mol

Solubility: Insoluble in water and organic solvents

Category: Glidant, anti-adherent

Description: A fine, white to grayish-white powder used to improve powder flow and reduce tablet sticking.

Uses: Talc is widely used in the pharmaceutical industry as a glidant, lubricant, and diluent in tablet and capsule formulations to improve powder flowability and prevent sticking during manufacturing. It acts as an anti-caking agent in powders and enhances the disintegration and dissolution of tablets for better drug absorption. Talc is also used as a coating agent in certain medications to provide a smooth finish and protect the drug from moisture. Additionally, it serves as a carrier for topical formulations like creams and dusting powders (Bazar et al., 2021; Jadhav et al., 2013).

2.2.7 Lactose

IUPAC Name: 4-O-β-D-Galactopyranosyl-D-glucopyranose

Molecular Formula: $C_{12}H_{22}O_{11}$

Molecular Weight: 342.30 g/mol

Solubility: It dissolves in water but does not dissolve in organic solvents.

Category: Diluent, binder

Description: A white crystalline powder used as filler in solid dosage forms.

Uses: Lactose is usually utilized in the pharmaceutical industry as a filler or diluent for tablet and capsule formulations because of its excellent compressibility, stability, and solubility. It acts as a carrier in dry powder inhalers (DPIs) for respiratory drugs and enhances the flow properties of powdered formulations. Additionally, lactose is used in lyophilized (freeze-dried) products and liquid formulations to improve drug stability and dispersion (Dominici et al., 2022; Zadow, 1984).

2.2.8 Microcrystalline Cellulose

IUPAC Name: Purified depolymerized cellulose

Molecular Formula: $C_6H_{10}O_5$

Molecular Weight: Variable

Solubility: Insoluble in water and organic solvents

Category: Binder, disintegrant, diluent

Description: A fine, white powder used as a multifunctional excipient in solid dosage forms.

Uses: It is usually utilized as an excipient in tablet and capsule preparations because of its superior binding, disintegrating, and bulking properties. It acts as a tablet binder and filler, ensuring uniform drug distribution and improving tablet strength while allowing for rapid disintegration. MCC also enhances flow properties in powder formulations, making it ideal for direct compression tableting. Additionally, it is used in sustained-release formulations and as a stabilizer in liquid and semi-solid dosage forms (Hindi and Hindi, 2016; Rana et al., 2022; Yohana Chaerunisaa et al., 2019).

2.3 Introduction to Hyperlipidemia

Hyperlipidemia is high blood lipid levels, raising the risk of heart attacks and strokes. The condition can be classified into primary (genetic) hyperlipidemia, caused by inherited disorders, and secondary (acquired) hyperlipidemia, which results from lifestyle factors, medical conditions, or medications (Onwe et al., 2015). The primary lipid components involved include LDL, HDL, VLDL and triglycerides (TG). High LDL and triglycerides contribute to plaque buildup in the arteries, leading to atherosclerosis, while high HDL is protective. Hyperlipidemia is identified through a lipid profile test that assesses total cholesterol, LDL, HDL, and triglyceride levels. Its management involves lifestyle changes, including a heart-healthy diet, regular physical activity, weight management, and quitting smoking.

In severe cases, medications like statins, fibrates, and PCSK9 inhibitors may be prescribed. If left untreated, hyperlipidemia can lead to heart disease, stroke, and other complications, making early detection and management essential (El Nabetiti et al., 2023; Shattat, 2014).

2.3.1 Cholesterol

Cholesterol is a lipid essential for cell membrane structure, hormone production, and digestion. Lipoproteins carry lipids in blood, causing plaque buildup and raising heart disease and stroke risk. Cholesterol comes from two sources: the liver (which produces most of it) and dietary intake from animal-based foods (Cabral and Klein, 2017). High cholesterol levels, often caused by poor diet, lack of exercise, obesity, or genetics, can be managed through lifestyle changes and medications like statins (Thongtang et al., 2022). Regular cholesterol screening is important for early

detection and prevention of heart-related complications. Cholesterol circulates in the bloodstream through various lipoproteins, which influence its impact on health. The major types of cholesterol include:

2.3.1.1 Total Cholesterol (TC)

Total cholesterol is the combined measurement of all cholesterol types in the blood, including LDL, HDL, and VLDL. It serves as a general indicator of lipid health, but evaluating individual components provides a more accurate risk assessment for cardiovascular disease. Total cholesterol under 200 mg/dL is healthy; 200–239 is borderline, 240+ is high and increases heart disease risk. Since LDL is harmful, HDL is protective, and VLDL contributes to triglycerides, the ratio of these lipoproteins is essential in determining overall health risks. High total cholesterol is influenced by diet, lifestyle, genetics, and medical conditions like diabetes and hypothyroidism, and it can be managed through dietary changes, exercise, weight control, and medications like statins if needed. Regular lipid profile testing is recommended to monitor and maintain healthy cholesterol levels (Peters et al., 2016).

2.3.1.2 Low-Density Lipoprotein (LDL)

LDL, or "bad cholesterol," carries cholesterol to cells. Excess LDL causes plaque buildup in arteries, raising the risk of heart attack, stroke, and artery disease (Ephraim et al., 2023). LDL cholesterol levels are classified as optimal (<100 mg/dL), near optimal (100-129 mg/dL), borderline high (130-159 mg/dL), high (160-189 mg/dL), and very high (≥190 mg/dL). Elevated LDL levels are influenced by diet high in saturated and trans fats, lack of physical activity, obesity, smoking, genetic factors, and medical conditions like diabetes and hypothyroidism. Managing LDL involves a heart-healthy diet (rich in fiber and healthy fats), regular exercise, weight control, and medications for example statins, PCSK9 inhibitors, and ezetimibe in high-risk cases. Regular cholesterol monitoring is essential for preventing cardiovascular complications (Tanaka et al., 2023).

2.3.1.3 High-Density Lipoprotein (HDL)

HDL, or "good cholesterol," removes excess cholesterol, helping prevent plaque buildup. Higher HDL reduces heart disease risk; ideal levels are 40+mg/dL for men, 50+ for women. Factors for example regular physical activity and a diet rich in healthy fats (e.g., olive oil, nuts, fatty fish), and smoking cessation can help boost

HDL levels, while obesity, a sedentary lifestyle, smoking, and excessive alcohol intake can lower it. Unlike LDL, having higher HDL is generally beneficial, but extremely high levels (above 100 mg/dL) may, in rare cases, have negative effects. Maintaining a balanced diet, active lifestyle, and healthy body weight is key to optimizing HDL levels and decreasing cardiovascular risk (Nessler et al., 2018; Zhou et al., 2015).

2.3.1.4 Very Low-Density Lipoprotein (VLDL)

VLDL is a type of lipoprotein that mainly transports triglycerides from the liver to different tissues throughout the body, where they are used for energy or stored as fat. Unlike LDL, which carries mostly cholesterol, VLDL contains a high percentage of triglycerides, making it a key player in fat metabolism. Excess VLDL can cause plaque buildup, increasing the risk of heart disease, stroke, and other complications (Heeren and Scheja, 2021). Although VLDL is not typically measured directly, its levels are estimated as one-fifth of triglyceride values, with normal VLDL levels being 2-30 mg/dL. High VLDL levels are often linked to obesity, insulin resistance, type 2 diabetes, excessive sugar and refined carbohydrate intake, alcohol consumption, and metabolic syndrome. Managing VLDL involves decreasing sugar and unhealthy fat intake, fetching in regular physical action, upholding a healthy weight, and, in severe cases, using medications for example fibrates, niacin, or omega-3 fatty acids (Balling et al., 2020). Keeping VLDL and triglyceride levels in check is essential for overall cardiovascular health.

2.3.1.5 Triglycerides (TG)

Triglycerides (TG) are a type of fat (lipid) found in the blood that serves as the body's primary energy storage. When you eat, excess calories-especially from carbohydrates and fats-are converted into triglycerides and stored in fat cells for later use. While triglycerides are essential for energy, high levels (hypertriglyceridemia) can contribute to cardiovascular disease, atherosclerosis, and pancreatitis (Laufs et al., 2020). Normal triglyceride levels are below 150 mg/dL, while 150-199 mg/dL is borderline high, 200-499 mg/dL is high, and 500 mg/dL or higher is very high. Elevated triglycerides are often associated with obesity, excessive sugar and alcohol intake, a sedentary lifestyle, insulin resistance, type 2diabetes, and metabolic syndrome. Lifestyle changes for example decreasing refined carbohydrates and

unhealthy fats, increasing physical activity, keeping a balanced weight, and incorporating omega-3s into the diet can help lower triglycerides. In severe cases, medications like fibrates, niacin, and prescription-strength omega-3 fatty acids may be needed to manage high triglyceride levels and reduce cardiovascular risks (Miller et al., 1998). Regular lipid profile testing is important for monitoring and maintaining healthy triglyceride levels.

2.3.2 Types of Hyperlipidemia

Hyperlipidemia is classified into different types based on the specific lipids that are elevated and the underlying causes. It is broadly categorized into

2.3.2.1 Primary (Genetic) Hyperlipidemia

Primary (Genetic) Hyperlipidemia is an inherited disorder caused by genetic mutations that affect lipid metabolism, leading to abnormally high levels of cholesterol, triglycerides, or together in the blood. It is classified under Frederickson's system, which includes

1. Type I – Familial Hyperchylomicronemia

Familial Hyperchylomicronemia is a rare genetic disorder characterized by extremely high levels of chylomicrons and triglycerides in the blood because of a deficiency or dysfunction of lipoprotein lipase (LPL) or its cofactor ApoC-II. This defect prevents the proper breakdown and clearance of chylomicrons, leading to triglyceride levels often exceeding 1000 mg/dL, which significantly upsurges the risk of acute pancreatitis. Other clinical features include eruptive xanthomas (yellowish skin lesions filled with fat), lipemia retinalis (milky appearance of blood vessels in the retina), hepatosplenomegaly (enlarged liver and spleen), and recurrent abdominal pain. Unlike other types of hyperlipidemia, Type I does not significantly increase the risk of atherosclerosis. Diagnosis is confirmed through lipid profile tests, genetic testing, and the presence of fasting chylomicronemia. Treatment focuses on a strict low-fat diet (<15% of daily calories from fat), avoidance of alcohol and simple carbohydrates, and supplementation with medium-chain triglycerides (MCTs) as an alternative energy source. Medications for example fibrates and statins are ineffective, making dietary management the primary approach to preventing complications like pancreatitis (Lourenco et al., 2024; Masson et al., 2023).

2. Type II – Familial Hypercholesterolemia (FH)

FH is an inherited condition marked by high levels of LDL cholesterol, which greatly raises the risk of early-onset atherosclerosis and cardiovascular disease. It is caused by mutations in the LDL receptor (LDLR) gene, ApoB-100 gene, or PCSK9 gene, resulting in defective LDL clearance from the bloodstream. FH is further classified into Type IIa (isolated high LDL) and Type Iib (high LDL and VLDL, often with elevated triglycerides). LDL cholesterol levels in FH patients are typically above 190 mg/dL in heterozygous FH (HeFH) and can exceed 400 mg/dL in the more severe homozygous FH (HoFH). Clinical features include tendon xanthomas (fat deposits in tendons, especially the Achilles), corneal arcus (cholesterol deposits around the cornea), and premature coronary artery disease (CAD), often before age 50 in men and 60 in women (Zubielienė et al., 2022). Diagnosis is based on lipid profile tests, genetic testing, and family history of early heart disease. Management includes aggressive lipid-lowering therapy, including statins, ezetimibe, PCSK9 inhibitors, and in severe cases, LDL apheresis. Lifestyle alterations for example a heart-healthy diet, regular exercise, as well as escaping smoking are indispensible for decreasing cardiovascular risks. Early detection and treatment are essential to prevent life-threatening complications (Fularski et al., 2024).

3. Type III – Familial Dysbetalipoproteinemia

Familial Dysbetalipoproteinemia (FD) is an inherited lipid disorder marked by the buildup of intermediate-density lipoproteins (IDL), chylomicron remnants, and VLDL remnants in the blood, leading to elevated total cholesterol and triglycerides (typically 300-600 mg/dL each). It is primarily instigated by a mutation in the Apolipoprotein E (ApoE) gene, particularly the ApoE2/E2 genotype, which disrupts the liver's ability to clear remnant lipoproteins. Individuals with FD are at a higher risk for early-onset atherosclerosis, coronary artery disease (CAD), peripheral artery disease (PAD), and cerebrovascular diseases for example stroke. Characteristic clinical signs include tuberoeruptive xanthomas and palmar xanthomas. Diagnosis is confirmed through lipid profile testing, ApoE genotyping, and the presence of remnant lipoproteins. Treatment focuses on lifestyle modifications for example a low-fat, low-sugar diet, regular physical activity, and weight management, along with lipid-lowering medications like fibrates, statins, niacin, and omega-3 fatty acids to reduce

triglyceride and cholesterol levels. Early detection and management are essential to prevent cardiovascular complications (Bea et al., 2023; Koopal et al., 2017).

4. Type IV – Familial Hypertriglyceridemia (FHTG)

FHTG is a genetic disorder characterized by elevated very VLDL and high triglyceride levels (typically between 200-500 mg/dL) because of increased hepatic VLDL production and impaired clearance. Unlike other forms of hyperlipidemia, LDL cholesterol is usually normal or slightly decreased, while HDL cholesterol is often reduced, increasing the risk of cardiovascular disease (Cruz-Bautista et al., 2021). The condition is polygenic, meaning it results from multiple genetic factors combined with lifestyle influences for example obesity, insulin resistance, excessive carbohydrate intake, alcohol consumption, and physical inactivity. Individuals with FHTG may remain asymptomatic, but in severe cases, extremely high triglyceride levels (>1000 mg/dL) can lead to acute pancreatitis, presenting with abdominal pain, nausea, and vomiting. Diagnosis is based on fasting lipid profile tests, family history, and the exclusion of secondary causes like diabetes or hypothyroidism. Management involves lifestyle modifications, including a low-carb and low-fat diet, regular exercise, weight loss, and avoiding alcohol and sugary foods. If triglycerides remain high despite lifestyle changes, medications for example fibrates, omega-3 fatty acids, and niacin may be recommended in the direction of lowering triglyceride levels and reduce the risk of complications (Parhofer and Laufs, 2019).

5. Type V – Mixed Hyperlipidemia

Mixed Hyperlipidemia is a genetic disorder characterized by elevated chylomicrons and VLDL, leading to extremely high triglyceride levels (often >1000 mg/dL) and moderate increases in total cholesterol. This condition results from a combination of genetic predisposition and secondary factors for example obesity, insulin resistance, diabetes, alcohol consumption, and a high-carbohydrate diet, which contribute to excessive triglyceride production and impaired lipid clearance. Unlike Type I hyperlipidemia, which only involves chylomicrons, Type V features both chylomicrons and VLDL, leading to a higher risk of pancreatitis, eruptive xanthomas (small, yellowish skin lesions), lipemia retinalis, and hepatosplenomegaly. The threat of atherosclerosis and CVD's is also improved, though less than in Type II hyperlipidemia. Diagnosis is based on fasting lipid profile tests, clinical symptoms,

and exclusion of secondary causes. Treatment includes a strict low-fat, low-carbohydrate diet, weight management, regular exercise, and avoiding alcohol and refined sugars. In severe cases, medications for example fibrates, omega-3 fatty acids, niacin, and statins (if LDL is elevated) are used, while patients with extreme hypertriglyceridemia may require hospitalization to prevent acute pancreatitis (Anton-Păduraru et al., 2014; Bello-Chavolla et al., 2018; Taghizadeh et al., 2019).

2.3.2.2 Secondary (Acquired) Hyperlipidemia

Secondary Hyperlipidemia is a lipid disorder caused by underlying medical conditions, lifestyle factors, or certain medications that disrupt normal lipid metabolism, leading to elevated levels of cholesterol, triglycerides, or both. Unlike primary hyperlipidemia, which is genetic, secondary hyperlipidemia is often reversible with proper treatment of the underlying cause (Yanai and Yoshida, 2021). Common medical conditions associated with secondary hyperlipidemia include diabetes mellitus (which increases VLDL and triglycerides), hypothyroidism (which reduces LDL clearance, leading to high LDL cholesterol), nephrotic syndrome (which raises LDL and total cholesterol), chronic kidney disease, and liver disorders for example non-alcoholic fatty liver disease (NAFLD). Lifestyle factors for example obesity, a high-sugar and high-fat diet, excessive alcohol consumption, smoking, and physical inactivity also contribute to dyslipidemia. Certain medications, including corticosteroids, beta-blockers, thiazide diuretics, oral contraceptives, antiretroviral drugs, and immunosuppressants, can elevate cholesterol and triglyceride levels. Diagnosis is based on lipid profile tests and identifying the underlying cause. Management focuses on treating the primary condition, adopting a heart-healthy diet, increasing physical activity, weight loss, and, if necessary, using lipid-lowering medications like statins, fibrates, omega-3 fatty acids, or niacin (Phogat et al., 2010). Addressing secondary hyperlipidemia is essential to decreasing the risk of atherosclerosis, cardiovascular disease, and pancreatitis.

2.3.3 Diagnosis of Hyperlipidemia

Diagnosis of Hyperlipidemia involves a combination of clinical evaluation, laboratory tests, and assessment of risk factors to determine lipid abnormalities and associated cardiovascular risks. The primary diagnostic tool is the fasting lipid profile (lipid panel), which measures TC, LDL-C, HDL-C, TG, and VLDL-C. A fasting lipid test is

typically conducted after 9-12 hours of fasting to ensure accurate triglyceride and LDL levels. Non-fasting lipid tests may also be used in some cases, especially for routine screening (El Nabetiti et al., 2023). Hyperlipidemia is diagnosed when LDL >130, total cholesterol >200, triglycerides >150, or HDL <40 (men) / <50 (women). Additional tests, for example Apolipoprotein B (ApoB), lipoprotein(a) [Lp(a)], and genetic testing, may be conducted in cases of suspected familial hyperlipidemia. A thorough medical history, family history of early heart disease, physical examination (checking for xanthomas, corneal arcus), and evaluation of secondary causes (for example diabetes, hypothyroidism, or liver/kidney disease) are also essential for accurate diagnosis (R. Saldanha et al., 2020). Cardiovascular risk assessment tools, for example the ASCVD Risk Calculator, may be used to determine the likelihood of heart disease and guide treatment decisions. Early and accurate diagnosis is essential for implementing lifestyle modifications and appropriate medical therapy to reduce cardiovascular complications (Azevedo et al., 2018).

2.3.4 Management & Treatment of Hyperlipidemia

The management and treatment of hyperlipidemia aim to reduce lipid levels, lower the risk of atherosclerosis, cardiovascular disease (CVD), and pancreatitis, and improve overall heart health. Treatment involves a combination of lifestyle modifications, pharmacological therapy, and management of underlying conditions.

Lifestyle modifications are the first-line approach to managing hyperlipidemia and decreasing the risk of CVD. A heart-healthy diet includes fruits, veggies, whole grains, lean proteins, and healthy fats, while limiting saturated fats, trans fats, and sugars. Engaging in regular physical activity, for example 150 minutes of moderate-intensity exercise per week—like brisk walking, jogging, or cycling—is beneficial for overall health, helps lower LDL and triglycerides while raising HDL. Weight management through a balanced diet and exercise reduces lipid levels and improves overall health. Smoking cessation is essential, as smoking lowers HDL and increases cardiovascular risk, while limiting alcohol intake helps prevent elevated triglycerides. Managing stress through meditation, yoga, or relaxation techniques also plays a role in overall cardiovascular health. These lifestyle changes, when consistently followed, can significantly lower lipid levels and reduce the need for medications (El Nabetiti et al., 2023; Ray, 2024).

2.3.5 Complications of untreated hyperlipidemia

Untreated hyperlipidemia can lead to serious cardiovascular and metabolic complications because of the accumulation of excess cholesterol and triglycerides in the bloodstream. One of the biggest risks is atherosclerosis, a condition in which fatty deposits accumulate in the arteries, leading to narrowing and a higher likelihood of developing CAD, heart attacks, and strokes (Won, 2023). Persistent high LDL cholesterol and low HDL cholesterol contribute to plaque formation, leading to hypertension, PAD, and carotid artery disease, which can cause reduced blood flow to the brain and limbs. Exceptionally higher TG levels (above 1000 mg/dL) can trigger acute pancreatitis, a life-threatening inflammation of the pancreas. Other complications include fatty liver disease (non-alcoholic fatty liver disease – NAFLD), kidney disease, and cognitive decline because of reduced blood supply to the brain. Individuals with untreated hyperlipidemia are at a higher risk of sudden cardiac death because of plaque rupture and blood clot formation. Early detection and proper management through lifestyle changes and medications are essential to prevent these life-threatening complications (Sniderman et al., 2014; Valeri et al., 1986).

2.4 Drugs used to treat Hyperlipidemia

Hyperlipidemia is usually treated with statins like atorvastatin and rosuvastatin, which lower LDL cholesterol by inhibiting HMG-CoA reductase. If statins are not tolerated or additional cholesterol reduction is needed, ezetimibe can be used to block cholesterol absorption in the intestine. For severe cases, PCSK9 inhibitors (e.g., alirocumab, evolocumab) help enhance LDL clearance. Fibrates for example fenofibrate and gemfibrozil are primarily used to lower triglycerides. Bile acid sequestrants (e.g., cholestyramine) and bempedoic acid offer alternative options for cholesterol reduction. In some cases, omega-3 fatty acids and niacin may also be used to manage triglyceride levels (Clebak and Dambro, 2020; S. Lad et al., 2023). Table 1 provides an outline of dissimilar lipid-lowering medications, including their dosages and potential side effects.

The 2024 review article published in *Current Problems in Cardiology* discusses the evolution of LDL-C lowering medications and their cardiovascular benefits. The study highlights statins, including lovastatin, as first-line agents in the management of hyperlipidemia. Lovastatin works by inhibiting HMG-CoA reductase, thereby

reducing cholesterol synthesis in the liver. The article reports that statins can reduce LDL-C levels by approximately 30–50%, depending on the dose and treatment intensity. Lovastatin, when administered as an oral tablet, effectively lowers total cholesterol and improves the overall lipid profile. The review emphasizes that statin therapy plays a crucial role in reducing the risk of major cardiovascular events. These include myocardial infarction, stroke, and other atherosclerotic complications. Furthermore, the study highlights the importance of long-term lipid control in preventing disease progression. Lovastatin demonstrates sustained efficacy when used over extended periods. The safety profile of lovastatin is also reported to be favorable, with minimal adverse effects in most patients. The article confirms that consistent LDL-C reduction is directly associated with improved cardiovascular outcomes. Overall, the findings reinforce that lovastatin remains a clinically significant and effective antilipidemic drug in modern therapeutic practice.

The study by Rafael Gamez, Sarahi Mendoza et al. aimed to compare the cholesterol-lowering effects and toxicity of D-003 and lovastatin in normocholesterolaemic rabbits. The experiment involved oral administration of D-003 at a dose of 5 mg/kg/day for 30 days. Lovastatin was administered at a higher dose of 10 mg/kg for comparison. Both treatments significantly reduced low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) levels. D-003 demonstrated a superior effect in increasing high-density lipoprotein cholesterol (HDL-C). In contrast, lovastatin was more effective in reducing triglyceride levels. These findings suggest that both agents have beneficial but slightly different lipid-modifying profiles. The study also evaluated the safety of D-003 at higher doses. Administration of higher doses for 10 days did not produce any observable toxicity related to D-003. This indicates a favorable safety profile for D-003 in the tested conditions. Overall, D-003 showed comparable efficacy to lovastatin in lowering cholesterol with additional benefits on HDL-C. The results highlight its potential as an alternative lipid-lowering agent with minimal toxicity.

The 2024 article published in *Molecules* explores the effect and mechanism of oleanolic acid in metabolic syndrome and cardiovascular diseases. The study highlights that oleanolic acid plays a significant role in regulating lipid metabolism. It influences key enzymes involved in cholesterol homeostasis, particularly Acyl-CoA:

Cholesterol Acyltransferase (ACAT). By inhibiting ACAT activity, oleanolic acid reduces the formation of cholesterol esters. This mechanism contributes to decreased lipid accumulation in tissues. The study reports that oleanolic acid significantly lowers triglycerides, low-density lipoprotein (LDL), and total cholesterol levels. It also improves overall lipid profile, thereby reducing the risk of cardiovascular diseases. In addition, oleanolic acid exhibits strong antioxidant properties, which help in reducing oxidative stress. Its anti-inflammatory effects further support metabolic balance and cardiovascular health. The compound also enhances hepatic lipid regulation, preventing excessive fat deposition in the liver. These combined actions make oleanolic acid an effective natural agent for managing dyslipidemia. The study confirms that its antilipidemic activity is partly mediated through ACAT inhibition. Overall, the findings support the therapeutic potential of oleanolic acid in the treatment of metabolic syndrome.

The study by Dong Won Jeong, Young Hoon Kim et al. investigated the pharmacokinetics of oleanolic acid following both intravenous and oral administration in rats. The oral doses evaluated were 10, 25, and 50 mg/kg to assess dose-linearity. At doses of 25 and 50 mg/kg, the pharmacokinetic parameters such as Tmax and half-life ($t_{1/2}$) were found to be comparable. The dose-normalized maximum plasma concentration (Cmax) ranged between 66 and 74 ng/mL when normalized to the 25 mg/kg dose. Similarly, the dose-normalized area under the curve (AUC) values were observed to be between 5.4 and 5.9 mg·min/mL for the same dose reference. These findings suggest a linear pharmacokinetic profile within the 25 to 50 mg/kg dose range. However, at the lower dose of 10 mg/kg, plasma concentrations were not measurable. This was primarily because the levels fell below the limit of quantitation, which was 2 ng/mL. The inability to detect the drug at this dose indicates poor systemic exposure at lower concentrations. It also highlights potential limitations in the oral bioavailability of oleanolic acid at sub-therapeutic doses. Overall, the study demonstrates that oleanolic acid exhibits dose-linear pharmacokinetics at moderate to higher doses. These findings are important for dose optimization and formulation development in future studies.

Table 1: Lipid-Lowering Drugs

Class	Examples	Dose (Typical Range)	Common Side Effects
Statins (HMG-CoA Reductase Inhibitors)	Atorvastatin, Rosuvastatin, Simvastatin, Pravastatin	10–80 mg/day 5–40 mg/day 10–40 mg/day	Muscle pain (myopathy), liver dysfunction, headache, GI disturbances
Cholesterol Absorption Inhibitor	Ezetimibe	10 mg/day	Diarrhea, fatigue, muscle pain, liver dysfunction
PCSK9 Inhibitors	Alirocumab, Evolocumab	75–150 mg SC every 15 days Evolocumab: 140 mg SC every 15 days or 420 mg per month	Injection site reactions, flu-like symptoms, neurocognitive effects
Fibrates (PPAR-α Agonists)	Fenofibrate, Gemfibrozil	Fenofibrate: 48–145 mg/day Gemfibrozil: 600 mg twice daily	GI disturbances, myopathy (especially with statins), liver dysfunction
Bile Acid Sequestrants	Cholestyramine, Colesevelam	4–16 g/day 3.75 g/day	Constipation, bloating, GI discomfort, reduced absorption of fat-soluble vitamins
Omega-3 Fatty Acids	Icosapent ethyl, Omega-3-acid ethyl esters	2 g twice daily 2–4 g/day	Fishy aftertaste, GI discomfort, increased bleeding risk
Niacin (Vitamin B3, Nicotinic Acid)	Niacin (extended-release or immediate-release)	500–2000 mg/day (gradual titration)	Flushing, itching, hepatotoxicity, hyperglycemia, GI upset

2.5 Combination therapy to treat Hyperlipidemia

Combination therapy is often used to treat hyperlipidemia when a single medication (monotherapy) is insufficient to achieve lipid targets, especially in high-risk patients. The most common combination involves statins with ezetimibe, where statins reduce cholesterol synthesis in the liver, while ezetimibe decreases intestinal cholesterol

absorption, providing an additive LDL-lowering effect (Masana et al., 2023). Another effective approach is combining statins with PCSK9 inhibitors (e.g., alirocumab or evolocumab), which enhance LDL receptor activity and significantly lower cholesterol levels, particularly in patients with familial hypercholesterolemia or statin intolerance. Statins and fibrates (e.g., fenofibrate or gemfibrozil) can be used together in cases of mixed dyslipidemia, helping lower LDL cholesterol while also decreasing triglycerides, though this combination requires caution because of an increased risk of myopathy (Wu et al., 2023). Similarly, omega-3 fatty acids can be added to statin therapy to further lower triglyceride levels without significant drug interactions (Barter and Ginsberg, 2008). Bile acid sequestrants, for example cholestyramine, can also be used alongside statins to enhance LDL cholesterol reduction by promoting bile acid excretion (Scaldaferri et al., 2013). In some cases, bempedoic acid is combined with statins for additional LDL-lowering benefits, especially in patients unable to tolerate high-dose statins (Natale et al., 2023). Combination therapy is tailored based on individual lipid profiles, cardiovascular risk, and potential drug interactions, ensuring optimal lipid control while minimizing adverse effects. The various combination therapies used to treat hyperlipidemia, including those containing ezetimibe, are summarized in Table 2. Advantages of combination therapies for hyperlipidemia are

Enhanced LDL Reduction – Combining drugs like statins and ezetimibe provides a greater reduction in LDL cholesterol than monotherapy alone.

Targeting Multiple Pathways – Different medications act through distinct mechanisms, for example inhibiting cholesterol synthesis (statins), decreasing cholesterol absorption (ezetimibe), or increasing LDL clearance (PCSK9 inhibitors), leading to more effective lipid control.

Better Triglyceride Management – Fibrates or omega-3 fatty acids combined with statins help lower triglycerides while maintaining LDL control, benefiting patients with mixed dyslipidemia.

Improved Cardiovascular Protection – Some combination therapies, for example rosuvastatin-ezetimibe-aspirin, not only lower lipids but also reduce the risk of blood clot formation, further protecting against heart disease and stroke.

Alternative for Statin-Intolerant Patients – Bempedoic acid-ezetimibe combinations offer LDL reduction for those who cannot tolerate statins because of muscle-related side effects.

Higher Treatment Adherence – Fixed-dose combination pills simplify the medication regimen, decreasing pill burden and improving patient compliance.

More Effective for High-Risk Patients – Individuals with familial hypercholesterolemia or existing cardiovascular disease benefit from combination therapies, which provide stronger lipid-lowering effects to meet stricter cholesterol targets.

Potential for Lower Doses and Fewer Side Effects – Using multiple agents at lower doses can reduce the risk of side effects associated with high-dose monotherapy, improving safety and tolerability.

Overall, combination therapies optimize lipid management, provide greater cardiovascular protection, and offer personalized treatment options for patients with varying lipid disorders.

Although several combination therapies have been reported for hyperlipidemia, there is limited inclusion of lovastatin-based combinations in prior literature. For example, colesevelam combined with lovastatin significantly reduced LDL cholesterol in hypercholesterolemic patients (Clin Cardiol. 2001), while low-dose lovastatin combined with niacin achieved similar efficacy as higher-dose lovastatin monotherapy, improving patient tolerability and adherence (Gardner SF, 1996). However, these studies did not explore fixed-dose combinations or compare pharmacokinetic profiles with other statin combinations such as simvastatin-ezetimibe or atorvastatin-fenofibrate. This indicates a research gap that the present study aims to address by evaluating novel lovastatin-based combination therapies, particularly in formulations designed for enhanced lipid-lowering efficacy and patient compliance.

Table 2: Combination therapies for Hyperlipidemia

Combination Therapy Name	Drug Components	Manufacturer
Polycap	Simvastatin (20 mg), Atenolol (50 mg), Ramipril (5 mg), Hydrochlorothiazide (12.5 mg), Aspirin (100 mg)	Cadila Pharmaceuticals
Crestor + ABT-335	Rosuvastatin (Crestor) + Fenofibric Acid (ABT-335)	AstraZeneca and Abbott
Atorvastatin + Torcetrapib	Atorvastatin (Lipitor) + Torcetrapib	Pfizer
Vytorin	Ezetimibe + Simvastatin	Merck/Schering-Plough
Liptruzet	Ezetimibe + Atorvastatin	Merck
Nexlizet	Ezetimibe + Bempedoic Acid	Esperion Therapeutics
Atozet	Ezetimibe + Atorvastatin	Multiple Manufacturers
Rosuvastatin + Ezetimibe + ASA	Rosuvastatin + Ezetimibe + Aspirin	Midas Pharma
Lovastatin + Colesevelam	Lovastatin + Colesevelam	Clin Cardiol. 2001
Lovastatin + Niacin	Lovastatin + Niacin	Gardner SF et al, 1996

2.6 Core and coat tablets

2.6.1 Introduction to Core and Coated Tablets

Core and coated tablets play a essential role in modern pharmaceutical formulations by enhancing drug stability, regulating drug release, and promoting patient adherence. The core tablet contains the active pharmaceutical ingredient (API) along with various excipients, while the coating layer serves different purposes, for example protecting the drug from environmental conditions, masking unpleasant taste, and providing

controlled or delayed drug release. Coated tablets are extensively used in DDS to improve therapeutic efficacy and ensure patient convenience (Tang et al., 2018).

2.6.2 Types of Tablet Coating

Tablet coatings are classified based on their function and composition:

1. Film Coating

Film-coated tablets are pharmaceutical dosage forms coated with a thin polymer layer to improve stability, appearance, and functionality. The coating, made from polymers like HPMC, ethyl cellulose, or methacrylate derivatives, provides protection against moisture, light, and environmental degradation, ensuring drug stability and prolonged shelf life. Film coating also masks unpleasant taste and odour, enhances swallowability, and can be designed for immediate, sustained, or enteric drug release. Compared to sugar coating, film coating is lighter, faster to apply, and maintains tablet size and weight. It is widely used in modern pharmaceuticals to enhance patient compliance, drug effectiveness, and formulation durability (Dasalkar and Munde, 2023).

2. Sugar Coating

Sugar coating is a conventional technique used in tablet formulation, where multiple layers of sugar-based solutions are applied to improve the tablet's appearance, taste, and stability. This process consists of several stages: sealing (to prevent moisture penetration), sub coating (to build tablet size and shape), smoothing, colouring, polishing, and printing (Ando et al., 2007). Common ingredients include sucrose, gelatin, acacia, calcium carbonate, and talc, which help form a thick, glossy, and aesthetically pleasing coat. Sugar coating effectively masks unpleasant tastes and odours, making tablets more palatable for patients. However, the process is time-consuming, labour-intensive, and increases tablet weight, making it less preferred compared to film coating, which provides a thinner, more efficient protective layer. Despite its limitations, sugar coating is still used in some pharmaceutical and confectionery applications where taste masking and an attractive finish are essential (Patil et al., 2024).

3. Enteric Coating

Enteric coating is a specialized tablet coating technique designed in the direction of preventing drug release in the acidic environment of the stomach and ensure

dissolution in the intestine (pH-dependent release). This coating protects acid-sensitive drugs from gastric degradation, minimizes gastric irritation, and enables targeted drug delivery for conditions like intestinal infections or inflammatory bowel disease (IBD). Enteric coatings are formulated with pH-sensitive polymers like Eudragit, cellulose acetate phthalate (CAP), and hydroxypropyl methylcellulose phthalate (HPMCP). These coatings stay intact in acidic environments but dissolve in the alkaline conditions of the small intestine. This technology is widely used for proton pump inhibitors (e.g., omeprazole), nonsteroidal anti-inflammatory drugs (NSAIDs), and pancreatic enzyme supplements, ensuring optimal drug absorption and therapeutic efficacy while decreasing side effects (Singh et al., 2022).

4. Compression Coating (core and coat)

Compression coating is a tablet coating technique where a dry outer coating layer is applied to a core tablet using a tablet compression process rather than traditional liquid-based coatings. This technique is predominantly beneficial for moisture-sensitive and heat-sensitive drugs since it eliminates the need for solvents or high temperatures. Compression coating allows for precise control over drug release, enabling immediate, sustained, or delayed-release formulations, including enteric-coated or pulsatile drug delivery systems. It also helps in separating incompatible drugs within the same tablet, improving stability and efficacy. Common excipients used in compression coating include cellulose derivatives, hydroxypropyl methylcellulose (HPMC), and polyethylene glycols (PEGs). This technique is widely applied in modified-release formulations, bilayer tablets, and fixed-dose combinations, ensuring better patient compliance and therapeutic benefits (Somnathe and Bhoyar, 2024; Vemula and Reddy, 2017).

5. Gastroretentive and pH-Sensitive Coatings

Gastroretentive and pH-sensitive coatings are advanced tablet coating technologies designed to prolong gastric retention and enable site-specific drug release based on pH conditions in the gastrointestinal (GI) tract. Gastroretentive coatings use floating, mucoadhesive, or expandable polymer systems to keep the tablet in the stomach for an extended period, improving the absorption of drugs with a narrow absorption window (e.g., metformin, levodopa). These coatings often incorporate gas-generating agents (e.g., sodium bicarbonate) or low-density polymers (e.g., HPMC, ethyl

cellulose) to maintain buoyancy in gastric fluids. pH-sensitive coatings, on the other hand, are formulated using enteric polymers like Eudragit, CAP, or HPMCP, which prevent drug release in the acidic stomach but allow dissolution in the alkaline intestine or colon (Nayak and Maji, 2014). These technologies are particularly useful for targeted drug delivery in gastric disorders, IBD, and delayed-release formulations, ensuring improved bioavailability, reduced side effects, and enhanced therapeutic outcomes.

2.6.3 Core Tablet Composition and Design

Core tablet composition and design are critical for ensuring optimal drug release, stability, and manufacturability. The core tablet typically consists of active pharmaceutical ingredients (APIs), excipients, and functional additives, each playing a specific role. Diluents (e.g., lactose, microcrystalline cellulose) provide bulk, while binders (e.g., starch, povidone) ensure tablet cohesion. Disintegrants (e.g., croscarmellose sodium) promote rapid breakdown, and lubricants (e.g., magnesium stearate) aid in manufacturing by decreasing friction. Depending on the intended drug release profile, core tablets can be designed for immediate, sustained, or controlled release using polymers and coating techniques. Special design features for example bilayer, multilayer, or compression-coated tablets enable drug combination therapies, taste masking, or targeted delivery. A well-optimized core tablet formulation ensures consistent drug efficacy, patient compliance, and large-scale production feasibility (Movva et al., 2013; Nagaraju et al., 2009).

2.6.4 Purpose and Benefits of Tablet Coating

The purpose of tablet coating is to enhance the stability, appearance, and functionality of pharmaceutical tablets while improving patient compliance and drug performance. Coating protects the active pharmaceutical ingredient (API) from moisture, light, and environmental degradation, extending the tablet's shelf life. It also masks unpleasant tastes and odours, making tablets easier to swallow (Arora et al., 2019). Additionally, coating enables controlled drug release, including immediate, sustained, or delayed (enteric) release, ensuring optimal therapeutic effects. It can also reduce gastric irritation, enhance mechanical strength, and provide brand identification through color or imprinting (Adepu and Ramakrishna, 2021). Overall, tablet coating improves drug

stability, efficacy, and patient experience, making it an essential aspect of modern pharmaceutical formulations.

2.6.5 Manufacturing Techniques for Tablet Coating

Manufacturing techniques for tablet coating involve various processes designed to apply a protective or functional layer over core tablets, enhancing stability, appearance, and drug release characteristics. The most common technique is film coating, where a polymer-based solution is sprayed onto tablets in a coating pan or fluidized bed system, followed by drying to form a thin, uniform layer. Sugar coating, an older technique, involves multiple layers of sugar-based solutions to mask taste and improve aesthetics but is time-consuming and increases tablet weight. Enteric coating utilizes pH-sensitive polymers to prevent drug release in the stomach, ensuring targeted intestinal absorption. Compression coating applies a dry outer layer via tablet compression, useful for moisture-sensitive drugs and modified-release formulations. Advanced techniques like electrostatic coating and hot-melt coating enhance efficiency and minimize solvent use. These techniques ensure consistent quality, controlled drug release, and improved patient compliance in pharmaceutical formulations (Akhtar Nehal Ahmed et al., 2021; Kumar et al., 2021).

2.6.6 Evaluation of core and coat tablets

Evaluation of core and coated tablets is essential to ensure quality, efficacy, and stability in pharmaceutical formulations. Core tablets undergo physicochemical tests for example hardness, friability, weight variation, and disintegration to assess their mechanical strength and uniformity. Drug content uniformity and *in vitro* dissolution studies determine dose accuracy and release profiles (Prasanthi et al., 2019). For coated tablets, additional evaluations include coating uniformity, adhesion, gloss, and moisture protection to ensure the coating remains intact and functional. Disintegration and dissolution tests assess whether the coating effectively controls drug release, especially for enteric or sustained-release formulations. Stability studies under accelerated conditions ensure long-term drug potency and shelf life (Shah et al., 2017). These tests collectively ensure that both core and coated tablets meet regulatory standards and deliver the intended therapeutic benefits.

2.6.7 Challenges and Limitations

The challenges and limitations of core and coated tablets primarily involve formulation complexity, manufacturing precision, and stability concerns. Core tablets must maintain uniform drug distribution, mechanical strength, and consistent disintegration, but issues like powder flow variability, compression force optimization, and friability can affect quality. Coated tablets face challenges in achieving uniform coating thickness, adhesion, and drying efficiency, as improper coating can lead to cracking, peeling, or delayed drug release. Moisture-sensitive coatings may degrade over time, impacting stability and shelf life. Additionally, coating process scalability, high production costs, and solvent-related safety concerns pose limitations for large-scale manufacturing (Somnathe and Bhoyar, 2024). Regulatory compliance requires rigorous testing to ensure bioavailability, dissolution profiles, and long-term effectiveness, making core and coated tablet development a technically demanding and resource-intensive process.

2.6.8 Recent Advances in core and coat tablets

Recent advances in core and coated tablets focus on enhancing drug delivery, stability, and manufacturing efficiency through innovative technologies. 3D printing enables precise control over drug layering, allowing for personalized medicine and complex release profiles. Nanocoatings and smart polymers improve targeted drug delivery, bioavailability, and controlled release, particularly in enteric and gastroretentive formulations. Electrostatic coating and hot-melt coating reduce solvent use, making the process safer and more environmentally friendly. Multiparticulate and multilayer tablet technologies allow for fixed-dose combinations, improving treatment outcomes for chronic diseases. Additionally, machine learning and AI-driven formulation optimization are being integrated to predict coating performance and drug release profiles, leading to more efficient and cost-effective production. These innovations significantly enhance the precision, functionality, and therapeutic effectiveness of core and coated tablets (Bajpai et al., 2012; Ganguly et al., 2022; Mandal, 2024).

2.6.9 Future Perspectives

Core and coated tablets play a vital role in modern pharmaceutical formulations by offering enhanced drug stability, controlled release, and improved patient compliance.

Advances in coating technology continue to drive the development of more effective and patient-friendly drug delivery systems. Future research may focus on bioresponsive coatings, personalized medicine applications, and the integration of nanotechnology for enhanced therapeutic outcomes (Zaid, 2020).

2.7 Bilayer tablets

2.7.1 Introduction to Bilayer Tablets

Bilayer tablets are pharmaceutical dosage forms composed of two distinct layers, each containing different active pharmaceutical ingredients (APIs) or release profiles. This technology enables controlled drug release, combining immediate and sustained-release actions in a single tablet. It is widely used in fixed-dose combination therapy (FDC) for chronic diseases like diabetes, hypertension, and pain management. The evolution of bilayer tablets reflects the growing demand for optimized drug release, improved patient compliance, and enhanced therapeutic efficacy (Kale et al., 2022).

2.7.2 Rationale for Bilayer Tablet Development

The development of bilayer tablets is driven by the need for improved drug release control and patient compliance. They enable the combination of immediate-release (IR) and sustained-release (SR) layers**,** ensuring a rapid onset of action while maintaining prolonged therapeutic effects. Bilayer formulations are particularly beneficial for fixed-dose combinations (FDCs)**,** allowing the co-administration of multiple drugs in a single tablet, which is essential for managing chronic conditions like diabetes, hypertension, and pain (Saikia and Sahu, 2017)**.** Additionally, bilayer technology helps separate chemically incompatible drugs**,** preventing undesired interactions while maintaining efficacy. They also facilitate targeted drug delivery**,** for example gastroretentive or mucoadhesive systems**,** improving drug absorption and bioavailability. Furthermore, bilayer tablets can reduce dose frequency**,** minimizing side effects and enhancing patient adherence to treatment regimens. Their versatility and efficiency make them a valuable innovation in modern pharmaceutical formulations (Deshpande et al., 2011).

2.7.3 Types of Bilayer Tablets

Bilayer tablets can be classified into different types based on their intended function:

1. Immediate-release & sustained-release combinations

Bilayer tablets combining IR and SR layers offer a dual drug delivery approach, enhancing therapeutic efficacy and patient compliance. The IR layer ensures a rapid onset of action by quickly dissolving and releasing the drug into systemic circulation, which is essential for conditions requiring immediate relief, for example pain, hypertension, or diabetes management. In contrast, the SR layer provides a controlled, prolonged release over several hours, maintaining steady drug levels and decreasing dosing frequency (Chauhan et al., 2012). This combination minimizes dose fluctuations, side effects, and drug resistance, making it ideal for chronic diseases requiring sustained therapy. By optimizing polymer selection, compression techniques, and dissolution profiles, IR-SR bilayer tablets improve bioavailability, patient adherence, and overall treatment outcomes (Mourya et al., 2023).

2. Combination of two different drugs

Bilayer tablets combining two different drugs offer a fixed-dose combination (FDC) approach, improving therapeutic effectiveness and patient convenience. This formulation allows for the simultaneous administration of drugs with different mechanisms of action, enhancing synergy and decreasing the pill burden. It is predominantly advantageous for treating chronic conditions like hypertension, diabetes, and pain management, where combining drugs improves efficacy while minimizing side effects. The bilayer design enables separation of chemically incompatible drugs, ensuring stability and controlled release. One layer can provide immediate action, while the other ensures sustained or delayed release, optimizing drug absorption and maintaining consistent plasma levels (Rameshwar et al., 2014). This approach enhances patient adherence, reduces dosing frequency, and improves treatment outcomes, making bilayer tablets an efficient and advanced drug delivery system.

3. Gastroretentive bilayer tablets

Gastroretentive bilayer tablets represent an advanced drug delivery system that extends gastric residence time, improving the absorption of drugs with a limited absorption window in the stomach or upper intestine. These tablets typically consist of a floating or mucoadhesive layer that helps retain the tablet in the stomach, while the second layer provides immediate or controlled drug release. The floating layer is

formulated using gas-generating agents (e.g., sodium bicarbonate) or low-density polymers (e.g., HPMC, ethyl cellulose), ensuring the tablet remains buoyant in gastric fluids. This system is particularly beneficial for drugs with poor solubility at higher pH levels, for example certain antibiotics, antifungals, and proton pump inhibitors. Gastroretentive bilayer tablets improve bioavailability, prolong therapeutic effects, and reduce dosing frequency, making them highly effective for treating gastric ulcers, Helicobacter pylori infections, and other gastrointestinal disorders (Dey et al., 2014; Maddiboyina et al., 2020).

4. Mucoadhesive bilayer tablets

Mucoadhesive bilayer tablets are an advanced drug delivery system designed to prolong drug retention at the site of absorption, enhancing bioavailability and therapeutic effectiveness. These tablets consist of two distinct layers: one mucoadhesive layer, which adheres to the mucosal surface (for example the gastrointestinal, buccal, or vaginal mucosa), and another drug-containing layer that provides either immediate or controlled drug release. The mucoadhesive layer is formulated using bioadhesive polymers like carbopol, HPMC, chitosan, or sodium alginate, ensuring prolonged contact with the absorption site (Patel et al., 2007). This system is especially beneficial for drugs with a short half-life, poor solubility, or extensive first-pass metabolism, as it enhances absorption and reduces dosing frequency. Mucoadhesive bilayer tablets are usually used in treating oral infections, gastric ulcers, hormone therapies, and localized drug delivery applications, improving patient compliance and therapeutic outcomes (Omer and Shahidulla, 2022).

5. pH-dependent bilayer tablets

pH-dependent bilayer tablets are a specialized DDS designed to release the drug at specific pH levels in the GIT, ensuring targeted drug delivery and improved therapeutic efficacy. These tablets consist of two layers: one that provides immediate or sustained release and another that is pH-sensitive, dissolving only at a particular pH. The pH-sensitive layer is formulated using enteric polymers like Eudragit, CAP, or HPMCP, which prevent drug release in the acidic stomach but allow dissolution in the alkaline environment of the small intestine or colon. This approach is especially beneficial for acid-sensitive drugs, colon-targeted therapies, and drugs with local action in the intestines, for example mesalamine for inflammatory bowel disease

(IBD). pH-dependent bilayer tablets enhance drug stability, reduce gastric irritation, and provide site-specific drug release, improving overall treatment efficiency (Rao et al., 2003; You et al., 2019).

2.7.4 Formulation Considerations

The formulation of bilayer tablets requires careful selection of excipients and process parameters to ensure stability, layer adhesion, and controlled drug release. Key considerations include choosing binders (e.g., HPMC, polyvinylpyrrolidone) to maintain layer integrity, disintegrants (e.g., sodium starch glycolate) for rapid drug release, and polymers (e.g., ethylcellulose, xanthan gum) to achieve sustained-release profiles. Proper compression force is essential to prevent layer separation, capping, or lamination, ensuring adequate mechanical strength. The use of lubricants (e.g., magnesium stearate) must be optimized to prevent tablet defects while maintaining flow properties. Drug-drug and drug-excipient compatibility studies are essential to avoid chemical instability. The formulation should also consider moisture sensitivity, solubility differences, and pH-dependent drug release. Additionally, the choice of granulation technique (direct compression, wet or dry granulation) significantly impacts the tablet's final properties. Proper optimization of these factors ensures the desired release kinetics, bioavailability, and patient compliance in bilayer tablet formulations (Singh et al., 2021).

2.7.5 Manufacturing Techniques

Bilayer tablets can be manufactured using different techniques, each with specific advantages and challenges:

1. Direct Compression

Direct compression is the simplest and most cost-effective technique for manufacturing bilayer tablets. In this process, the powder blends for each layer are directly compressed into tablets without the need for granulation. This technique requires excipients with good flowability and compressibility to ensure uniform layer formation. However, direct compression poses challenges for example layer separation, capping, and poor adhesion between layers because of the lack of binding agents. Optimizing compression force and using appropriate binders can help improve layer integrity and mechanical strength (Gafitanu et al., 2015).

2. Dry Granulation

Dry granulation is used when drugs are sensitive to moisture or heat, making wet granulation unappropriate. In this technique, the drug-excipient mixture is first compacted into slugs or roller-compacted ribbons, which are then milled into granules and compressed into bilayer tablets. This technique enhances tablet density, flowability, and layer adhesion, decreasing the risk of layer separation. However, dry granulation requires high compression forces, which can sometimes cause excessive hardness and reduced drug dissolution rates (Mahata et al., 2022).

3. Wet Granulation

Wet granulation involves mixing the drug and excipients with a liquid binder to form wet masses, which are then dried, milled, and compressed into tablets. This technique improves powder cohesion, layer uniformity, and mechanical strength, making it ideal for bilayer tablets where strong adhesion between layers is necessary. However, wet granulation involves additional processing steps, longer drying times, and higher production costs (Patel et al., 2015). It is not appropriate for drugs that are moisture-sensitive or have poor stability in liquid media.

2.7.6 Challenges and Limitations

Bilayer tablets pose several challenges and limitations in their formulation and manufacturing. One of the primary issues is layer separation (delamination), which occurs because of poor adhesion between layers, often caused by inadequate compression force or incompatible excipients. Capping and lamination are also common problems, resulting from air entrapment or excessive compression pressure. Additionally, dissolution variability can arise when the two layers have different solubilities, leading to inconsistent drug release profiles. Drug-drug and drug-excipient incompatibility may also affect the stability and efficacy of the formulation, requiring extensive compatibility studies. Manufacturing complexities, for example precise control over compression parameters and selection of appropriate binders and lubricants, add to the production cost and time. Furthermore, scalability issues may arise, as ensuring uniformity and reproducibility in large-scale manufacturing is challenging (Blicharski et al., 2019). Despite these limitations, continuous advancements in tablet technology, excipient selection, and compression techniques are helping to improve the quality and feasibility of bilayer tablet production.

2.7.7 Evaluation Parameters for Bilayer Tablets

Bilayer tablets must undergo rigorous testing to ensure quality and efficacy:

1. Physicochemical evaluation

Physicochemical evaluation of bilayer tablets ensures their structural integrity, uniformity, and mechanical strength. Key tests include weight variation, thickness, hardness, friability, and layer adhesion to confirm tablet uniformity and durability during handling. Tensile strength testing assesses the bond between layers to prevent delamination or layer separation. Additionally, drug content uniformity and disintegration testing verify accurate dosing and proper breakdown of the immediate-release layer for optimal therapeutic effect (Panda et al., 2015).

2. *In vitro* dissolution studies

In vitro dissolution studies for bilayer tablets assess the drug release profile from both layers under simulated gastrointestinal (GI) conditions. The test is conducted using a USP dissolution apparatus (Paddle or Basket technique) in different pH media to mimic stomach and intestinal environments. The immediate-release (IR) layer is expected to dissolve rapidly, typically within 30–60 minutes, ensuring a quick onset of action. In contrast, the sustained-release (SR) layer is designed to release the drug gradually over 6–12 hours to maintain therapeutic levels. Mathematical models for example zero-order, first-order, Higuchi, and Korsmeyer-Peppas are used to analyze drug release kinetics, helping to understand the mechanism of drug diffusion and release. These studies help in optimizing formulation design, ensuring regulatory compliance, and predicting *in vivo* drug performance (Ryakala et al., 2015).

3. Stability studies

Stability studies of bilayer tablets are conducted to evaluate their shelf life, drug integrity, and physical stability under various environmental conditions. These studies follow ICH (International Council for Harmonisation) guidelines, which include accelerated stability testing (at 40°C ± 2°C and 75% ± 5% RH) for 6 months and long-term stability testing (at 25°C ± 2°C and 60% ± 5% RH) for up to 24 months. The tablets are periodically analyzed for physical appearance (color changes, layer separation, hardness), drug content, dissolution profile, and chemical degradation. Any significant variation in these parameters may indicate instability, requiring formulation modifications. Stability testing ensures that bilayer tablets retain their

efficacy, safety, and quality throughout their intended shelf life, preventing dose variations or therapeutic failures (Solakhia et al., 2012).

2.7.8 Advances and Innovations in Bilayer Tablet Technology

Advances in bilayer tablet technology focus on enhancing drug release control, stability, and patient compliance through innovative formulation and manufacturing techniques. Advanced compression technologies, for example dual rotary tablet presses with precise force control, improve layer adhesion and prevent delamination. The use of novel polymers and nanotechnology-based excipients enables better sustained-release profiles and bioavailability enhancement. 3D printing and hot-melt extrusion have emerged as cutting-edge approaches to design complex, personalized bilayer formulations with tailored drug release kinetics. Additionally, floating and mucoadhesive bilayer tablets enhance gastroretention for drugs with narrow absorption windows. These innovations contribute to more efficient, patient-friendly, and commercially viable bilayer tablet formulations, expanding their therapeutic applications (Ratnaparkhi and Ganesh, 2014).

2.7.9 Regulatory Aspects and Market Trends

The regulatory aspects of bilayer tablets are governed by agencies like the U.S. FDA, EMA, and ICH, which set stringent guidelines for quality, safety, efficacy, and manufacturing practices. Regulatory approval requires extensive preclinical and clinical studies, stability testing, *in vitro* dissolution profiling, and bioequivalence studies to ensure consistent drug release and therapeutic performance. Compliance with Good Manufacturing Practices (GMP) and Quality by Design (QbD) principles is essential to minimize variability and enhance product reliability. Market trends indicate a growing demand for bilayer tablets in chronic disease management, particularly in cardiovascular, diabetes, and pain management therapies, because of their ability to combine multiple drugs and optimize release profiles. The rise of fixed-dose combinations (FDCs), innovative drug delivery systems, and 3D printing technologies is further driving the commercial expansion and acceptance of bilayer tablets in the pharmaceutical industry (Agiba et al., 2021; Crișan et al., 2023).

2.7.10 Research gaps and novelty

Although extensive research has explored various drug classes and combination therapies for hyperlipidemia, most studies focus primarily on pharmacological

efficacy, with limited attention to optimized tablet formulations for improved patient compliance and controlled drug release. Similarly, while advances in core, coated, and bilayer tablet technologies have been reported, few studies investigate their integration specifically for hyperlipidemia combination therapy. In particular, the development of bilayer tablets combining statins and ezetimibe with advanced coating techniques, such as compression or pH-sensitive coatings, remains underexplored. The present study aims to address these gaps by formulating such bilayer tablets to achieve enhanced LDL reduction, sustained and targeted drug release, and improved patient adherence, representing a novel approach in lipid-lowering therapy and modern drug delivery systems.

3. HYPOTHESIS

This study hypothesizes that the combination of lovastatin and oleanolic acid will provide superior therapeutic efficacy in the management of hyperlipidemia compared to monotherapy. Lovastatin, a well-established HMG-CoA reductase inhibitor, effectively reduces cholesterol synthesis and lowers LDL levels, whereas oleanolic acid, a bioactive phytoconstituent, exhibits antihyperlipidemic, antioxidant, and hepatoprotective properties. The complementary mechanisms of action of these two agents are expected to produce a synergistic effect, improving lipid profile, reducing oxidative stress, and protecting hepatic function, thereby enhancing overall therapeutic outcomes.

However, both drugs suffer from poor oral bioavailability and rapid metabolism, which may limit their clinical effectiveness. To overcome these limitations, a modified-release drug delivery system is proposed. It is further hypothesized that a bilayer and core–coat tablet formulation can be designed to achieve both immediate and sustained drug release. The immediate release of lovastatin, facilitated by superdisintegrants such as sodium starch glycolate and croscarmellose sodium, is expected to provide rapid onset of lipid-lowering action. In contrast, sustained release of oleanolic acid using a coating system composed of HPMC K100 and ethyl cellulose is anticipated to maintain prolonged therapeutic levels.

Furthermore, varying the ratio of HPMC K100 to ethyl cellulose in the coating layer is expected to significantly influence drug release kinetics, bioavailability, and formulation stability. This optimized polymeric system is hypothesized to enhance pharmacokinetic consistency and improve the overall efficacy of the combination therapy. Therefore, the proposed formulation strategy is expected to offer a more effective and controlled antilipidemic treatment compared to conventional dosage forms.

4. AIM AND OBJECTIVES

4.1 Aim

Development and Evaluation of Modified Release Tablets of Lovastatin and Oleanolic acid

4.2 Objectives

1. Formulation and evaluation of bilayer and core and coat tablets of lovastatin and oleanolic acid
2. Comparison of *in vitro* release from prepared tablets and selection of appropriate formulation based on optimum release.
3. Stability study of optimized formulation as per ICH guidelines
4. Pharmacokinetic and Pharmacodynamic evaluation of optimized formulation

5. MATERIALS:

5.1 Materials

Table 3: List of materials used in study

Sr. No.	Chemicals	Manufacturers
1.	Lovastatin	Lupin Pharmaceutical Ltd, Mumbai
2.	Oleanolic acid	Tokyo Chemical Industry Co. Ltd., Tokyo, Japan
3.	Hydroxypropyl Methylcellulose K100	S. D. Fine Chemicals, Mumbai
4.	Ethyl Cellulose	Loba Chemie Pvt. Ltd., Mumbai, India
5.	Sodium Starch Glycolate	
6.	Croscarmellose Sodium	
7.	Magnesium Stearate	
8.	Talc	
9.	Lactose	
10.	Microcrystalline Cellulose	
11.	Methanol	Merck Life Science Pvt. Ltd., Mumbai, India
12.	Acetonitrile	
13.	O-Phosphoric Acid	
14.	Sodium Lauryl Sulfate) solution	Loba Chemie Pvt. Ltd., Mumbai, India
15.	Benorilate	Merck Life Science Pvt. Ltd., Mumbai, India

5.2. Equipment

Table 4: List of equipment used in the study

Sr. No.	Equipments	Model/Manufacturer
1.	Electronic Weighing Balance	AY-120, Shimadzu Co. Ltd., Kyoto, Japan
2.	Differential Scanning Calorimeter	DSC-60, Shimadzu Co. Ltd., Kyoto, Japan
3.	X-ray Diffractometer	Bruker Corporation, Billerica, USA
4.	Fourier Transform Infrared (FTIR) Spectrophotometer	Bruker Corporation, Billerica, USA
5.	Reversed-Phase High-Performance Liquid Chromatography	JASCO Corporation Hachioji, Tokyo
6.	UV-Vis Spectrophotometer	UV 1780, Shimadzu Co. Ltd., Kyoto, Japan
7.	Sonicator	Prama Instruments Pvt. Ltd., Mumbai, India
8.	Water Purification System	UHQ-II, ELGA LabWater, United Kingdom
9.	Tablet Compression Machine	Karnavati Engineering Limited, Gujarat, India
10.	Dissolution Apparatus	Electrolab (India) Pvt. Ltd., Mumbai, India
11.	Disintegration Apparatus	
12.	Hardness Tester –	Bexco Exports, Punjab,India
13.	Friability Tester	Rolex Scientific Engineers, Haryana, India
14.	Stability Chamber	Remi Elektrotechnik Limited, Mumbai, India
15.	Liquid Chromatography-Mass Spectrometry (LC-MS/MS) System	API 2000, Shimadzu Co. Ltd., Kyoto, Japan
16.	Sieves	Sieve No. 100, Bhushan Engineering & Scientific Traders, Ambala, India

6. EXPERIMENTAL WORK

6.1 Physical Properties

6.1.1 Color Observation

The color of the lovastatin (LV) and oleanoic acid (OA) samples was observed visually. The samples were placed on a white background to accurately determine its color. The colors were recorded as white to off-white based on visual inspection.

6.1.2 State Determination

The physical state of the LV and OA samples was assessed by examining its texture and appearance. The sample was handled carefully to observe its form. Both the drugs were identified as a crystalline powder based on its solid, powdery appearance.

6.1.3 Melting Point Determination

A small amount of the LV and OA sample was finely ground to ensure uniformity. Thiele's apparatus was used for the determination of the melting points. An open capillary tube technique was engaged for the determination of the melting points of lovastatin and oleanolic acid (Awofisayo et al., 2010).

6.2 Fourier Transform Infrared (FTIR) Spectroscopy Analysis:

The FTIR spectrum of LV, OA and different composition of drug - polymers was recorded in the range of 4000-400 cm^{-1} to capture the full spectrum of functional groups and molecular vibrations. The FTIR spectrophotometer was calibrated according to the manufacturer's instructions prior to analysis. The FTIR spectrometer collected data in absorbance mode, and the resulting spectrum was analyzed to identify characteristic peaks corresponding to various functional groups present in lovastatin. The recorded FTIR spectrum was analyzed to identify characteristic peaks and functional groups based on their absorption frequencies. The positions of the peaks and their intensities were compared with standard IR absorption data to confirm the presence of specific functional groups in the lovastatin sample (Naeem et al., 2010).

6.3 Differential Scanning Calorimetry (DSC) Analysis

The drug sample was accurately weighed and sealed in aluminum pans to prepare for DSC analysis. Each pan was hermetically closed to prevent any loss of sample during heating. The instrument used was Shimadzu-60 DSC instrument. The samples were subjected to a heating program with a constant rate of 10°C/min. The analysis was

conducted under a dry nitrogen flow at a rate of 100 mL/min to provide an inert atmosphere and to prevent oxidation. The temperature was scanned from 25°C to 350°C to cover the expected range of thermal events for the sample. The presence of a clear and sharp endothermic peak indicates melting point of samples (Ebrahimi et al., 2024).

6.4 X-ray Diffraction (XRD) Analysis

The crystalline nature of the drug sample was characterized expending XRD analysis to assess its purity and structural properties. The analysis was performed with an X-ray diffractometer equipped with Cu Kα radiation. For the study, the LV and OA samples was finely ground and carefully placed into a sample holder designed for XRD measurements. The XRD patterns were recorded at a scanning rate of 1°/min over a diffraction angle (2θ) range of 10° to 80°. This range was selected to encompass the angles where significant crystallographic peaks were anticipated. The resulting XRD spectra revealed sharp and intense peaks, indicating a highly crystalline structure of the drug sample (Phadnis et al., 1997). These distinct and prominent peaks are characteristic of the crystalline nature of API, which is essential for understanding its physical and chemical properties.

6.5 Analytical method Development

For the simultaneous estimation of lovastatin (LV) and oleanolic acid (OA), a comprehensive set of instruments was employed to ensure precision and accuracy. The analysis was conducted using High-Performance Liquid Chromatography (HPLC) with Borwin chromatography software (version 1.50) for data acquisition. A Model PU 2080 Plus Intelligent HPLC pump delivered the mobile phase, and samples were injected via a Rheodyne sample injection port with a 20 µL loop. The separation was achieved on a SunQSil C18 column, and detection was performed using a JASCO UV-2075 UV-VIS detector. Additionally, a Shimadzu Double Beam Spectrophotometer (Model UV 1780) was utilized for UV-Vis spectrophotometric analysis. Precise measurements were ensured with a Shimadzu Electronic Weighing Balance (Model AY-120), and a Prama Solutions Sonicator facilitated efficient dissolution and mixing of samples. High-purity water, essential for the analysis, was obtained using an Elga Lab (PURELAB UHQ-II) water purification system, and calibrated glassware was used for all volume measurements. The mobile phase

selection was a critical aspect of developing a reliable technique for the simultaneous estimation of LV and OA. Initial trials using acetonitrile (ACN) and methanol in a 50:50 (v/v) ratio did not provide adequate separation, as indicated by an insufficient number of theoretical plates. Various compositions were subsequently tested, leading to the optimized mobile phase of methanol (MeOH) and water, adjusted to pH 3 with o-phosphoric acid in a 90:10 (v/v) ratio. This composition significantly improved the theoretical plates and produced well-defined peaks for both analytes, meeting system suitability parameters and ensuring accurate and reliable simultaneous estimation of lovastatin and oleanolic acid (Sabir et al., 2016; Savadkouhi et al., 2017).

6.6 Technique validation

6.6.1 Preparation of quality control standards

Standard stock solutions were prepared to establish a calibration curve for the simultaneous estimation of lovastatin and oleanolic acid. For oleanolic acid, 10 mg of the compound was precisely weighed and dissolved in 10 mL of MeOH, resulting in a standard stock solution with a concentration of 1000 µg/mL. Similarly, 10 mg of lovastatin was dissolved in 10 mL of MeOH to create a standard stock solution with a concentration of 1000 µg/mL. From these standard stock solutions, working standard solutions were prepared by diluting the standard stock solutions with MeOH to achieve a concentration of 100µg/mL for both oleanolic acid and lovastatin. Working solutions for the RP-HPLC analysis were prepared from the 100 µg/mL standard stock solutions.

6.6.2 Selection of Detection Wavelength

To determine the optimal detection wavelength for simultaneous estimation, the standard stock solutions of oleanolic acid and lovastatin were further diluted with methanol and scanned over the wavelength range of 200-400 nm.

6.6.3 Specificity

The specificity of the technique was determined by analyzing blank samples, standard solutions of lovastatin and oleanolic acid, and their mixture.

6.6.4 System Suitability

System suitability tests were conducted to ensure that the HPLC system was operating correctly. This involved evaluating parameters for example retention time, peak shape, and theoretical plates. A series of six replicate injections of a standard solution

containing lovastatin and oleanolic acid were performed, and the % RSD for retention time, peak area, and theoretical plates were calculated (Sharma et al., 2018).

6.6.5 Linearity

Linearity was assessed by preparing standard solutions of lovastatin and oleanolic acid at various concentrations within the analytical range (2-12 µg/mL). Calibration curves were generated by plotting peak areas against their respective concentrations.

6.6.6 Accuracy

Accuracy was evaluated by spiking known quantities of lovastatin and oleanolic acid into the matrix and analyzing the spiked samples. The percentage recovery of both drugs was determined by comparing the measured concentration with the known added concentration. Validation was conducted through recovery studies at three concentration levels: 50%, 100%, and 150% of the target concentration, with three replicates each, and calculating the % RSD for the recoveries to ensure precision and consistency (Mulholland et al., 1991).

6.6.7 Precision

Precision was evaluated by assessing repeatability (intra-day precision) and intermediate precision (inter-day precision). Repeatability was evaluated by analyzing six replicate injections of a standard solution containing lovastatin and oleanolic acid on the same day, and the % RSD for the peak areas was calculated (Kowalska et al., 2022). Intermediate precision was assessed by analyzing the same standard solution on three different days and calculating the % RSD for the peak areas.

6.6.8 Robustness

Robustness was tested by deliberately varying the chromatographic conditions for example flow rate (0.95,1,1.05 mL/min), mobile phase composition of Methanol and Water (92:8, 90:10 and 88:12 v/v), and wavelength (209,201,211 nm). The impact on the separation and quantification of lovastatin and oleanolic acid was evaluated by analyzing standard solutions under these varied conditions. The standard solution was injected three times for each varied conditions and its effect on peak area, recovery and retention time was examined and recorded (Raut and Shaji, 2021).

6.6.9 Estimation of Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the slope of the calibration curve (S) and the standard deviation of the response

(sigma), with the standard deviation of the Y-intercepts of the regression line serving as the standard deviation (Nugrahani and Dillen, 2018).

$$LOD = \frac{3.3\ \sigma}{S} \quad \text{Eq. (1)}$$

$$LOQ = \frac{10\ \sigma}{S} \quad \text{Eq. (2)}$$

6.7 Formulation of core and coat tablets

The first step of formulation of core and coat tablets involves preparation of core tablets. The core tablets of OA were prepared in 9 batches (OA1-OA9). Optimized batch amongst these batches were finalized based on dissolution studies. The optimized core tablet is used to prepare core and coat tablets. Different formulations of coating powder blend (LV1 to LV9) were used along with optimized core tablet to prepare core and coat tablets.

6.7.1 Formulation of core tablets

A 3^2 full factorial design was employed to explore the impact of two factors (HPMC K100 and Ethyl Cellulose) at three different levels. This design allowed for an assessment of the interactions between these factors and their impact on the drug release characteristics. The independent variables for this study were concentration of HPMC K100 (X1) and Ethyl cellulose (X2) whereas the dependent variables were maximum percentage release of drug at 2h (Y1) and maximum percentage release of drug at 12h (Y2) of core tablets. Experimental design and formulation design of core tablets are shown in table 5 and 6 respectively. Sustained release core layer of oleanolic acid was prepared by direct compression technique by adding required quantities of drug and other ingredients like HPMC K100, Ethyl Cellulose, Micro crystalline cellulose, and Lactose. Weigh accurately all the excipients and drugs and pass-through sieve no. 100. Later magnesium stearate and talc were added. The above mixture was compressed into Rimek tablet compression machine (Make Karnavati) B tooling using 8 mm concave punch.

6.7.2 Characterization of core tablets Formulation

The characterization of the granules involved assessing various physical properties to ensure the suitability of the formulations for tablet manufacturing. The parameters

evaluated included bulk density (BD), tapped density (TD), Hausner ratio (HR), Carr's Index (CI), and the angle of repose.

6.7.2.1 Bulk Density

BD was determined by carefully pouring a measured quantity of granules into a 100 mL graduated cylinder without tapping. The volume occupied by the granules was recorded, and BD was calculated using the formula,

$$Bulk\ Density = \frac{Mass\ of\ granules}{Volume\ of\ granules} \quad \text{Eq. (3)}$$

Table 5: Experimental design of core tablet

Factor	Low Level (mg)	Middle Level (mg)	High Level (mg)
HPMC K100 (X1)	10	15	20
Ethyl Cellulose (X2)	10	15	20

Table 6: Formulation design of core and coat tablets

Batch No.	Ingredients (mg)							
	Oleanolic Acid	HPMC	Ethyl Cellulose	Magnesium Stearate	Talc	Lactose	Micro Crystalline Cellulose	Total Weight
OA 1	50	10	10	2	3	12.5	12.5	100
OA 2	50	10	15	2	3	10	10	100
OA 3	50	10	20	2	3	7.5	7.5	100
OA 4	50	15	10	2	3	10	10	100
OA 5	50	15	15	2	3	7.5	7.5	100
OA 6	50	15	20	2	3	5	5	100
OA 7	50	20	10	2	3	7.5	7.5	100
OA 8	50	20	15	2	3	5	5	100
OA 9	50	20	20	2	3	2.5	2.5	100

6.7.2.2 Tapped Density

TD was measured by mechanically tapping a graduated cylinder containing a known mass of granules until no further volume change was observed. The TD was then calculated using the formula:

$$Tapped\ Density = \frac{Mass\ of\ granule}{Tapped\ volume\ of\ granules} \quad \text{Eq. (4)}$$

6.7.2.3 Hausner Ratio

The HR, an indicator of flow properties, was determined by dividing the TD by the BD:

$$Hausner\ Ratio = \frac{Tapped\ Density}{Bulk\ Density} \quad \text{Eq. (5)}$$

6.7.2.4 Carr's Index

Values of CI's below 15% generally indicate good flowability and compressibility, which are beneficial for tablet production. CI's was calculated using the formula:

$$Carr's\ Index = \frac{Tapped\ Density - Bulk\ Density}{Tapped\ Density} \times 100 \quad \text{Eq. (6)}$$

6.7.2.5 Angle of Repose

The angle of repose, which provides insight into the flow characteristics of granules, was measured by allowing the granules to flow through a funnel and form a conical pile. The angle formed by the pile was measured, with lower angles indicating better flow properties (Nagarani and Radha, 2023).

6.7.2.6 Tablet Hardness

To determine the mechanical strength of the tablets, their hardness was measured using a Monsanto hardness tester. This device applies a diametric force to the tablet using an inbuilt spring, with the hardness expressed in kg/cm². A single tablet was positioned between the jaws of the hardness tester, and the force needed to break it was measured and recorded (Battu et al., 2017). This procedure was repeated for three tablets from each batch to ensure consistency and reliability of the results.

6.7.2.7 Tablet Friability

The friability of the tablets, which measures their ability to withstand mechanical abrasion during handling and transport, was determined using a Rolex friabilator. A sample of tablets was weighed accurately and placed in the friabilator drum, which was operated at a speed of 25 rpm for 4 minutes, completing 100 revolutions. After

rotation, the tablets were removed, dusted, and reweighed (Chandani et al., 2012). The percentage weight loss was calculated using the formula:

$$Friability\ (\%) = \frac{Initial\ weight - Final\ weight}{Initial\ weight} \times 100 \qquad \text{Eq. (7)}$$

6.7.2.8 Dissolution study of Core Tablets

Dissolution testing was performed using a Type II (paddle) apparatus (Electrolab) with 900 mL of 0.1% SLS in water at 37 ± 0.5°C, stirred at 100 rpm. Samples (10 mL) were withdrawn at specified intervals up to 12 hours, replaced with fresh medium, filtered, diluted with mobile phase, and analyzed by HPLC. The percentage of drug release was determined using a linear equation, and the optimized batch was selected based on the highest drug release within 12 hours.

6.7.3 Preparation of coating blend tablet

A 3^2 full factorial design similar to that described in Section 6.7.3 was employed. Experimental design and formulation design of coating blend are shown in table 7 and 8 respectively. Different formulations LV1 to LV9 were formulated by using varying ratios of SSG and crosscarmellose sodium. The immediate-release layer of lovastatin was formulated using the direct compression technique. The required amounts of the drug and excipients, including SSG, MCC, and Lactose, were accurately weighed and passed through a sieve (#100). Subsequently, magnesium stearate and talc were incorporated into the mixture. The final blend was then compressed into tablets using a Rimek tablet compression machine (Karnavati) with B tooling, employing an 8 mm concave punch.

Table 7: Experimental design of coating blend

Factor	Low Level (mg)	Middle Level (mg)	High Level (mg)
Sodium Starch Glycolate (X1)	4	8	12
Croscarmellose sodium (X2)	4	8	12

6.7.4 Characterization of coating blend tablet

The characterization of the coating blend involved assessing various physical properties to ensure the suitability of the formulations for tablet manufacturing. The parameters evaluated included BD, TD, HR, CI's, and the angle of repose

(Fristiohady et al., 2021). Also post formulation study of prepared SR layer was performed by using different parameters like Hardness test, friability test etc.

6.7.5. Dissolution study of coating blend tablet

The dissolution test was performed on Dissolution testing apparatus (Electrolab, Type II- Paddle Type) using 900 mL volume of 0.1% SLS solution in water as dissolution media at 37 ± 0.5°C and 100 rpm speed.

6.7.6 The disintegration test (DT) of coat layer

The disintegration test was performed in water at 37°C, six tablets were inserted in each tube of DT apparatus (Electrolab). The apparatus was operated and time required for last tablet to disintegrate was noted (Kute et al., 2023).

Optimized formulation from batches LV1-LV9 was selected depending on maximum percentage of drug release at 30 min. and minimum disintegration time.

6.7.7 Preparation of core and coat tablets

The mixture of optimized formulation batch of coating blend was used in preparation of outer coat. The required mixture of polymers as per the optimized formulation (LV9) was weighed and mixed in is mortar. Tableting was performed by using is tableting machine by using 12 mm flat punches. One half of the Polymer mixture was added into the die, to make in powder bed, in the center of which an optimized core tablet (OA1) was placed. Then the remaining half of the mixture was added in the die and the total contents were compressed, thus producing in finished impress core and coat tablet (Khan et al., 2023).

Table 8: Formulation design of core and coat tablets

Batch No.	Ingredients (mg)							
	Lovastatin	Sodium Starch Glycolate	Croscarmellose sodium	Magnesium Stearate	Talc	Lactose	MCC	Total Weight
LV 1	10	4	4	4	6	86	86	200
LV 2	10	4	8	4	6	84	84	200
LV 3	10	4	12	4	6	82	82	200
LV 4	10	8	4	4	6	84	84	200
LV 5	10	8	8	4	6	82	82	200
LV 6	10	8	12	4	6	80	80	200
LV 7	10	12	4	4	6	82	82	200
LV 8	10	12	8	4	6	80	80	200
LV 9	10	12	12	4	6	78	78	200

6.7.8 Evaluation of core and coat tablets

Evaluation of core and coat tablet was performed by using different parameters like Hardness test, friability test etc.

6.7.9 Dissolution study of core and coat Tablets

Dissolution studies were performed using the general method described in Section 6.7.5 (Soni et al., 2008).

6.8 Formulation of Bilayer tablets

The first step of formulation of bilayer tablets involves preparation of sustained release (SR) layer. The SR layers of BOA were prepared in 9 batches (BOA1-BOA9). Optimized batch amongst these batches were finalized on the basis of dissolution studies. The optimized SR layer is used to prepare bilayer tablets. Different formulations of immediate release layer (BLV1 to BLV9) were used along with optimized SR layer to prepare bilayer tablets.

6.8.1 Formulation of SR layer of bilayer tablets

A 3^2 full factorial design was employed to explore the influence of two factors (HPMC K100 and Ethyl Cellulose) at three different levels. This design allowed for an assessment of the interactions between these factors and their impact on the drug release characteristics. The independent variables for this study were concentration of HPMC K100 (X1) and Ethyl cellulose (X2) whereas the dependent variables were maximum percentage release of drug at 2h (Y1) and maximum percentage release of drug at 12h (Y2) of SR layer (Pradeep Kumar et al., 2021). Experimental design of SR layer of bilayer tablet is similar as per the experimental design of core tablet which is shown in table 5 and formulation design of SR layer of bilayer tablets are shown in table 9. The sustained-release layer was prepared by direct compression using the same procedure described in Section 6.7.3, with polymer composition varied as per Table 9 (Shwetha Margret JL and Madhavi BLR, 2019).

6.8.2 Characterization of SR layer of bilayer tablets

Powder characterization was carried out for the SR/IR layer blends using bulk density, tapped density, Carr's index, Hausner's ratio, and angle of repose, as described in Section 6.7.4

6.8.3 Dissolution of SR layer of bilayer tablets

Dissolution studies were performed using the general method described in Section

6.7.5 (Soni et al., 2008).

6.8.4 Preparation of IR layer of bilayer tablets

A 3^2 full factorial design similar to that described in Section 6.7.3 was employed (Sarfaraz et al., 2020). Experimental design of IR layer blend of bilayer tablet is similar as per the experimental design of coating blend of core and coat tablet which is shown in table 7 and formulation design of IR layer blend of bilayer tablets are shown in table 10.

Table 9: Formulation design of SR layer of bilayer tablets

Batch No.	Ingredients (mg)							
	Oleanolic Acid	HPMC K100	Ethyl Cellulose	Magnesium Stearate	Talc	Lactose	Micro Crystalline Cellulose	Total Weight
BOA1	50	10	10	2	3	12.5	12.5	100
BOA2	50	10	15	2	3	10	10	100
BOA3	50	10	20	2	3	7.5	7.5	100
BOA4	50	15	10	2	3	10	10	100
BOA5	50	15	15	2	3	7.5	7.5	100
BOA6	50	15	20	2	3	5	5	100
BOA7	50	20	10	2	3	7.5	7.5	100
BOA8	50	20	15	2	3	5	5	100
BOA9	50	20	20	2	3	2.5	2.5	100

6.8.5 Preparation of IR layer of bilayer tablets

A 3^2 full factorial design similar to that described in Section 6.7.3 was employed. Experimental design of IR layer blend of bilayer tablet is similar as per the experimental design of coating blend of core and coat tablet which is shown in table 5 and formulation design of IR layer blend of bilayer tablets are shown in table 10. Different formulations BLV1 to BLV9 were formulated by using varying ratios of SSG and CS. The immediate-release layer of bilayer tablets was prepared by direct compression as described in Section 6.7.3, using formulations BLV1–BLV9. (Table 10)

6.8.6 Characterization IR layer of bilayer tablets

Powder characterization was carried out for the SR/IR layer blends using bulk density, tapped density, Carr's index, Hausner's ratio, and angle of repose, as described in Section 6.7.4

Table 10: Formulation design of IR layer of bilayer tablets

Batch No.	Ingredients (mg)							
	Lovastatin	SSG	CS	Magnesium Stearate	Talc	Lactose	MCC	Total Weight
BLV1	10	4	4	4	6	86	86	200
BLV2	10	4	8	4	6	84	84	200
BLV3	10	4	12	4	6	82	82	200
BLV4	10	8	4	4	6	84	84	200
BLV5	10	8	8	4	6	82	82	200
BLV6	10	8	12	4	6	80	80	200
BLV7	10	12	4	4	6	82	82	200
BLV8	10	12	8	4	6	80	80	200
BLV9	10	12	12	4	6	78	78	200

6.8.7 Dissolution study of IR layer of bilayer tablets

Dissolution studies were performed using the general method described in Section 6.7.5 (Soni et al., 2008).

6.8.8 The disintegration test of IR layer of bilayer tablets

The disintegration test was performed in water at 37° C, six tablets were inserted in each tube of DT apparatus (Electrolab). The apparatus was operated and time required for last tablet to disintegrate was noted.

Optimized formulation from batches BLV1-BLV9 was selected depending on maximum percentage of drug release at 30 min. and minimum disintegration time.

6.8.9 Preparation of Bilayer tablets

The optimized batch BOA1 was chosen based on the dissolution behavior of the sustained-release layer, while batch BLV9 was selected for the immediate-release layer considering its dissolution profile and disintegration time. To prepare the bilayer tablets, the sustained-release layer containing oleanolic acid was first placed in the die cavity and compressed with low force. Thereafter, the immediate-release layer of lovastatin was added to the die cavity and compressed with optimal hardness to form the final bilayer tablets (Israr et al., 2022; Shirse, 2012). The bilayer tablets were formulated using a Rimek tablet compression machine (Karnavati) with B tooling, employing a 12 mm concave punch.

6.8.10 Evaluation of Bilayer tablets

Evaluation of bilayer tablet was performed by using different parameters like Hardness test, friability test, Disintegration test etc.

6.8.11 Dissolution study of Bilayer tablets

Dissolution studies were performed using the general method described in Section 6.7.5 (Soni et al., 2008).

6.9 Drug Release Kinetics study

To determine the release kinetics of optimized formulations the *in vitro* drug release data were evaluated using different kinetic models to determine the drug release mechanism and behavior. The models applied included Zero Order, First Order, Higuchi, and Korsmeyer-Peppas (Akhter et al., 2012; Dash et al., 2010; Nagarwal et al., 2010; Solanki and Motiwale, 2020).

6.9.1 Zero Order release model

For the Zero Order release model, the drug release at various time intervals was plotted against time. This model assumes a steady release rate that remains unaffected by drug concentration and is expressed by the following equation.

$$Q = Q_0 + k_0 t \qquad \text{Eq. (8)}$$

Where, Q is the amount of drug released, Q_0 is the initial drug amount, and k_0 is the zero-order release constant. The linearity of the plot provided the zero-order release constant.

6.9.2 First Order release model

The First Order release model was used to assess whether the release rate is proportional to the drug concentration.

$$\log C = \log C_0 - \frac{Kt}{2.303} \qquad \text{Eq. (9)}$$

Where, C represents the drug concentration at time t, C_0 is the initial concentration, and K is the first-order rate constant. The logarithm of the concentration versus time plot was used to determine the first-order rate constant.

6.9.3 Higuchi model

In the Higuchi model, which describes release from a matrix system based on the square root of time?

$$Q = k_H t^{\frac{1}{2}} \qquad \text{Eq. (10)}$$

Here, Q is the amount of drug released, and k_H is the Higuchi release constant. The amount of drug released was plotted against the square root of time, with the slope representing k_H.

6.9.4 Korsmeyer-Peppas model

The Korsmeyer-Peppas model was employed for complex release mechanisms, using the equation

$$\frac{Q_t}{Q_\infty} = K_k t^n \qquad \text{Eq. (11)}$$

Where, $\frac{Q_t}{Q_\infty}$ denotes the fraction of drug released at $K_k t^n$ time is the release rate constant, and *n* is the release exponent. A log-log plot of the fraction of drug released versus time allowed for the determination of the release exponent and rate constant.

6.10 Stability Studies

Stability testing was conducted to assess how the quality of the formulated tablets varies over time when exposed to environmental factors like temperature, humidity, and storage conditions. This testing is essential for monitoring the drug's degradation over time and ensuring its effectiveness and safety. The optimized batch of core and coat and bilayer tablet were kept for stability studies for six months at 40 ± 2ºC/75 ± 5% relative humidity in a stability chamber (Remi Electrotechnik, Mumbai, India). The changes in their physical appearance, average weight of tablets, hardness, drug content and friability were observed (Ahmed et al., 2012; Al-Gohary and Al-Kassas, 2000; Venugopalarao et al., 2013).

6.11 Pharmacodynamic study

6.11.1 Experimental Animals

Healthy New Zealand white rabbits (2-3 kg) of either sex were used for this study. Each animal was housed separately in stainless steel cages and kept under standard laboratory conditions. They were given either a normal diet or a high-fat diet, along with unrestricted access to water (Wilson et al., 2023). The experimental protocol was approved by the institutional animal ethics committee, and all procedures were conducted in accordance with the Committee for the Control and Supervision of Experiments on Animals (CCSEA) guidelines.

6.11.2 Procedure

All animals were acclimatized to laboratory conditions prior to the experiments. On the day of the experiment, the animals were fasted overnight. Lipid profiles were performed, and only animals with normal lipid profiles were selected for the study.

6.11.3 Selection of Doses

For Oleanolic Acid

- Human dose: 50 mg
- Average weight of human: 60 kg
- Dose for 1 kg of human: 50 mg / 60 kg = 0.83 mg/kg
- Conversion factor for rabbit: 3.2
- Dose for rabbit: 0.83 mg/kg × 3.2 = 2.65 mg/kg

For Lovastatin

- Human dose: 10 mg

- Average weight of human: 60 kg
- Dose for 1 kg of human: 10 mg / 60 kg = 0.16 mg/kg
- Conversion factor for rabbit: 3.2
- Dose for rabbit: 0.16 mg/kg × 3.2 = 0.52 mg/kg

6.11.4 Experimental Design

The rabbits were divided into various groups (n=6) as follows:

- Group I: Normal control group
- Group II: High-fat diet control group
- Group III: High-fat diet group administered Lovastatin (API) 0.52 mg/kg
- Group IV: High-fat diet group administered Bilayer Tablet (containing Oleanolic Acid 2.65 mg, Sustained Release and Lovastatin 0.52 mg, Immediate Release)
- Group V: High-fat diet group administered core and coat Tablet (containing Oleanolic Acid in Core (2.65 mg, Sustained Release) and Lovastatin in coat (0.52 mg, Immediate Release). The grouping and numbering of experimental animals are described in table 11.

6.11.5 Induction of Hyperlipidemia in Rabbits

Rabbits were fed a standard basal diet for 2 weeks for adaptation. Group I was fed a normal standard diet, while the remaining groups were fed a hypercholesterolemic diet (1% cholesterol) for 2 weeks. Following this period, rabbits were administered their respective treatments for 2 weeks (Wang et al., 2013). After the last treatment, the body weight of all animals was recorded. Blood samples were collected on the last day of treatment, and serum was separated by centrifugation at 3000 RPM at 10°C for 20 minutes. The separated serum was used for the estimation of various lipid profiles.

6.11.6 Estimation of Lipid Parameters in Serum

The lipid parameters, including TC, HDL-C, LDL-C, and TG, were estimated using an autoanalyzer.

Table 11: Grouping and Numbering of Animals

Sr.no.	Grouping	Description	Animal code
1	Group 1 Normal control	Saline	G1/1
			G1/2
			G1/3
			G1/4
			G1/5
			G1/6
2	Group 2 Disease control	High Fat Diet (HFD)	G2/1
			G2/2
			G2/3
			G2/4
			G2/5
			G2/6
3	Group 3 Standard	HFD + Lovastatin (API) (0.5 mg/kg)	G3/1
			G3/2
			G3/3
			G3/4
			G3/5
			G3/6
4	Group 4 Test compound A	HFD + Oleanolic Acid and Lovastatin Bilayer Tablet	G4/1
			G4/2
			G4/3
			G4/4
			G4/5
			G4/6
5	Group 5 Test compound B	HFD + core and coat Tablet (containing Oleanolic Acid in Core (2.65 mg) and Lovastatin in coat (0.52 mg)	G5/1
			G5/2
			G5/3
			G5/4
			G5/5
			G5/6

6.12 Pharmacokinetic study

6.12.1 Preparation of Stock Solutions of Drugs

Stock solutions for Lovastatin and Oleanolic Acid were prepared by dissolving 10 mg of each drug in methanol, followed by dilution to 10 mL with methanol in a 10 mL

volumetric flask, yielding concentrations of 1000 µg/mL. Working stock solutions were prepared by diluting 1 mL of this stock solution to 10 mL with methanol, resulting in a concentration of 100 µg/mL. Further, a 1 µg/mL stock solution was made by diluting this working stock solution with methanol to a final volume of 100 mL. For Lovastatin, a 0.1 µg/mL solution was prepared by further dilution.

6.12.2 Preparation of Stock Solution of Internal Standard (IS)

Benorilate (IS) stock solution was prepared by dissolving 10 mg of Benorilate in methanol, then diluting to 10 mL with methanol in a 10 mL volumetric flask to obtain a concentration of 1000 µg/mL. A working solution of the internal standard was prepared by pipetting 0.1 mL of the IS stock solution (100 µg/mL) into a 100 mL volumetric flask and diluting to the final volume with the mobile phase, resulting in a concentration of 1 µg/mL.

6.12.3 Preparation of Spiked Plasma Samples

For the preparation of spiked plasma samples, 0.1 mL of plasma was added to each glass tube along with stock solutions of the standard drugs (as per Table 1) and 0.05 mL of the IS (10 µg/mL). The samples were vortex-mixed for 10 minutes, followed by the addition of a respective volume of methanol for protein precipitation to achieve a final volume of 1 mL. The mixture was vortexed again for 10 minutes and subsequently centrifuged for 20 minutes at 1500 rpm. Post-centrifugation, 10 µl aliquots of the supernatant from each tube were injected into the LC-MS/MS system for analysis. The various concentrations of spiked plasma samples are shown in table 12.

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Table 12: Composition of spiked plasma samples

Plasma	Stock solutionof LV	Stock solutionof OA	Stock solution of BN (IS) (1 µg/mL)	Methanol (Protein precipitation)	Conc. of LV (ng/mL)	Conc. of OA (ng/mL)	Conc.of BN(IS) (ng/mL)
0.1 mL	-	-	-	0.9 mL	-	-	-
0.1 mL	-	-	0.05 mL	0.95 mL	-	-	50
0.1 mL	0.01 mL	0.1 mL	0.05 mL	0.74 mL	1	100	50
0.1 mL	0.02 mL	0.2 mL	0.05 mL	0.63 mL	2	200	50
0.1 mL	0.04 mL	0.3 mL	0.05 mL	0.51 mL	4	300	50
0.1 mL	0.06 mL	0.4 mL	0.05 mL	0.39 mL	6	400	50
0.1 mL	0.08 mL	0.5 mL	0.05 mL	0.27 mL	8	500	50
0.1 mL	0.1 mL	0.6 mL	0.05 mL	0.15 mL	10	600	50

6.12.4 Instruments and Chromatographic Conditions

The pharmacokinetic evaluation was conducted using an API 2000 LC-MS/MS system, which included a Shimadzu LC 20ADvp pump, a Shimadzu SIL-HTc auto-sampler, and a Luna C18 100A analytical column (100 × 4.6 mm, 3.5 μm). Data acquisition and quantitation were managed by the Applied Biosystems "Analyst" version 1.4.1 software. The Turbo Ion Spray® interface was used, with the mass spectra obtained using the API source in positive ion mode (Gao et al., 2012). The system operated in multiple reaction monitoring (MRM) mode for all analytes (Lovastatin and Oleanolic Acid) and the internal standard (Benorilate).

The mobile phase consisted of a mixture of methanol and 0.1% formic acid in an 80:20 ratio, operating at a flow rate of 0.6 mL/min. The cone voltage settings were 135 V for Lovastatin, 125 V for Oleanolic Acid, and 125 V for Benorilate, while the capillary voltage was maintained at 2500 V. Argon was used as the collision gas, with collision energies of 20 eV for Lovastatin, 15 eV for Oleanolic Acid, and 10 eV for Benorilate. During tuning, the gas flow was optimized at 6 L/min, with the nebulizer pressure set at 30 psi. The source temperature remained at 320°C.

6.12.5 Pharmacokinetic Analysis

Blood samples (0.3 mL) were drawn using blood vessel cannulation from the marginal ear vein or jugular vein and collected in 1.5 mL microtubes pre-treated with 15 μL of heparin. Sampling was performed at specific time points: 0 (before drug administration), 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 10, 16, 24, and 48 hours post-dose. The collected samples were centrifuged at 1500 rpm for 15 minutes to separate plasma, which was then stored at −70°C until further analysis. Prior to analysis, samples were thawed to room temperature and examined using LC-MS/MS.

7. RESULTS AND DISCUSSION:

7.1 Physical properties

7.1.1 Colour Observation

The lovastatin sample was observed to be white to off-white in color. The oleanolic acid sample was observed to be white to light yellow in color.

7.1.2 State Determination:

Both the drugs lovastatin and oleanolic acid was found to be in a crystalline powder form.

7.1.3 Melting Point Determination

The melting point of lovastatin was found to be in the range of 171-174°C. The melting point of oleanolic acid was found tobe in the range of 305-308°C.

7.2 FTIR Spectroscopy Analysis

The FTIR spectrum of lovastatin was recorded in the range of 4000-400 cm^{-1} using an infrared spectrophotometer. The analysis revealed the presence of several characteristic peaks (Fig. 3), which are indicative of the functional groups within the lovastatin molecule. The observed peaks and their corresponding assignments are 3015.4 cm^{-1} (C=C stretching), 3537.2 cm^{-1} (O-H stretching), 1215.1 cm^{-1} (C-O-C stretching), 1054.8 cm^{-1} (C-O stretching), 1379.1 cm^{-1} (C-H bending), 2963.2 cm^{-1} (C-H stretching). These peaks correspond to the functional groups present in the lovastatin molecule, confirming its identity and purity. The results of the FTIR study are consistent with the known spectrum of lovastatin, indicating that the sample used in this study is of appropriate quality for further formulation development.

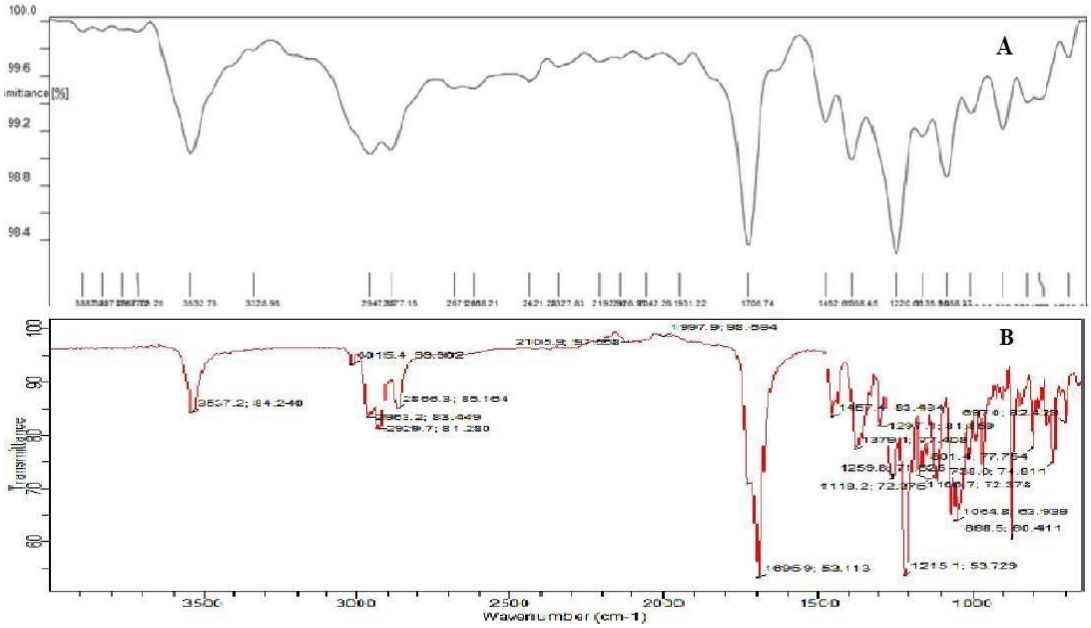

Fig. 3: FTIR Study of A) Reported Lovastatin B) Pure Drug Lovastatin

The FTIR spectrum of oleanolic acid was recorded in the range of 4000-400 cm^{-1} using an infrared spectrophotometer. The analysis revealed the presence of several characteristic peaks, which are indicative of the functional groups within the oleanolicacid molecule. The observed peaks and their corresponding assignments are 1427.37 cm^{-1} (C=C stretching), 3495.13 cm^{-1} (O-H stretching), 1597.11 cm^{-1} (C-O stretching), 3117.07 cm^{-1} (C-H stretching). These peaks correspond to the functional groups present in the oleanolic acid molecule, confirming its chemical structure and purity. The FTIR results (Fig. 4), ensure that the oleanolic acid sample used in this study is consistent with its expected chemical profile, which is essential for further formulation and evaluation.

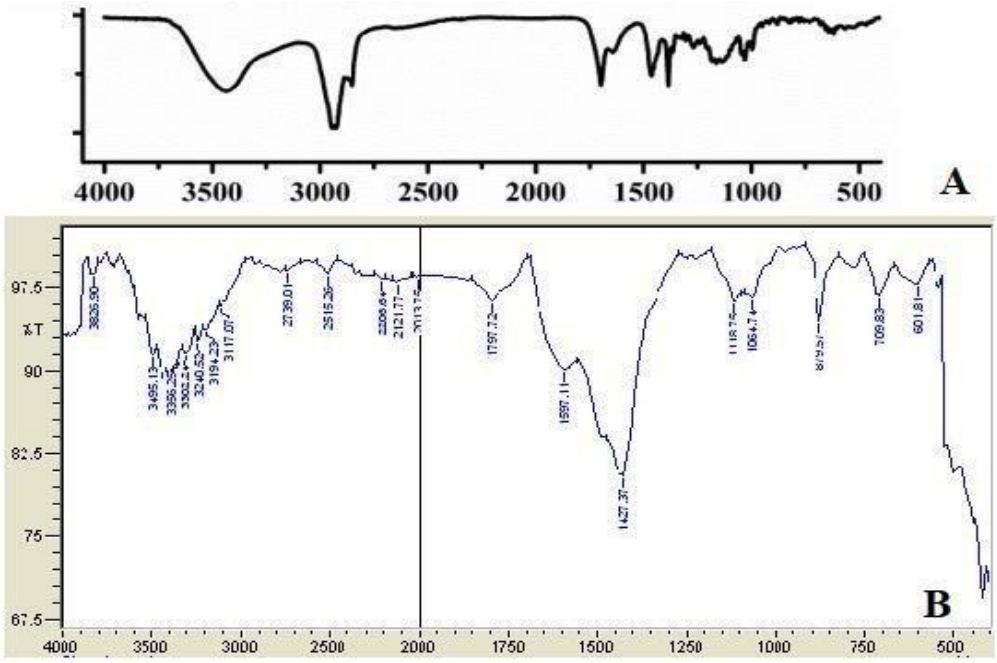

Fig. 4: FTIR Study of A) Reported Oleanolic Acid B) Pure Drug Oleanolic acid

7.3 Differential Scanning Calorimetry (DSC) Analysis

The consistency of the melting point observed in the DSC study (Fig. 5) with the previously determined melting point (171-174°C) further confirms the reliability and reproducibility of the thermal analysis. This characterization is essential for understanding the thermal behavior of lovastatin, which is essential for its formulation into stable and effective modified release tablets.

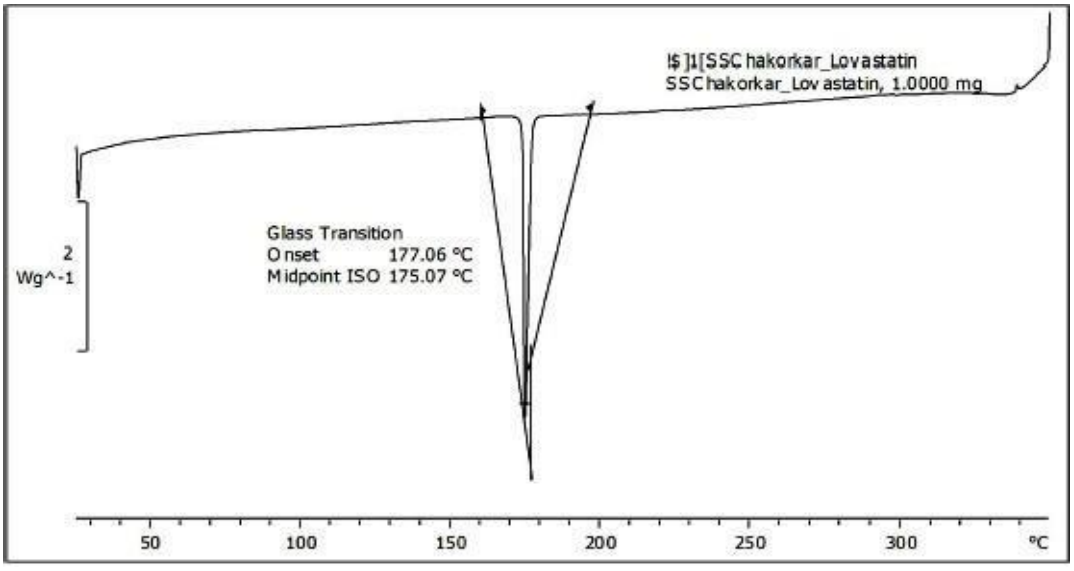

Fig. 5: DSC Thermogram of Lovastatin

The DSC thermogram of the pure oleanolic acid sample exhibited a distinct melting endotherm at 314.5ºC. This thermal event corresponds to the melting point of oleanolic acid, corroborating the results obtained from the melting point determination by the capillary technique. The presence of a sharp endothermic peak at this temperature (Fig. 6) indicates the purity and crystalline nature of the oleanolic acid sample used in the study.

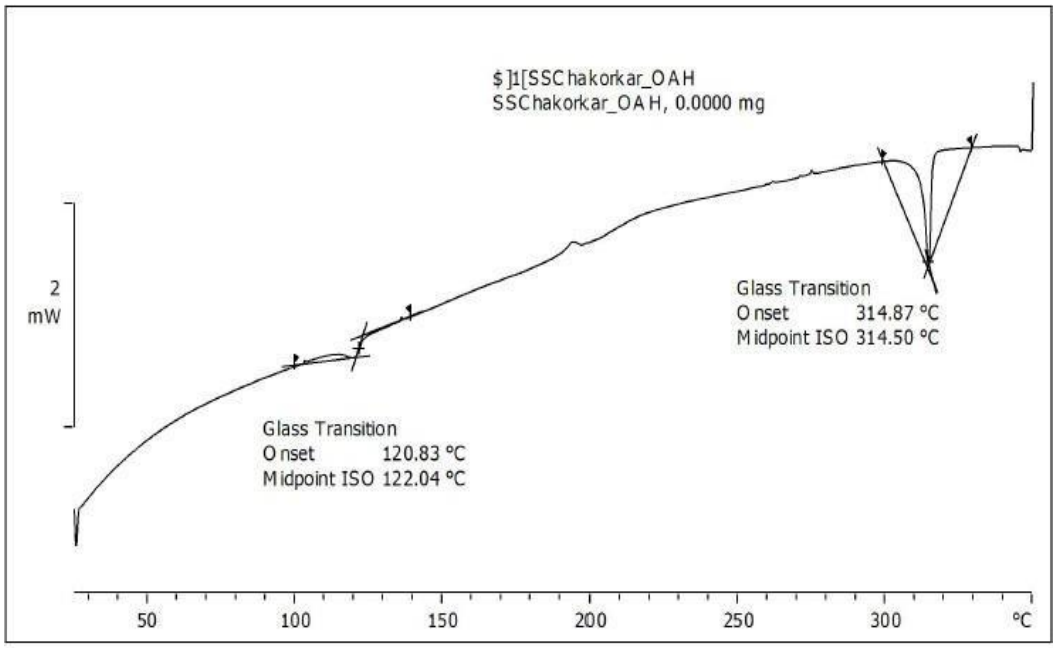

Fig. 6: DSC Study of Oleanolic acid

7.4 X-ray Diffraction (XRD) Analysis

The XRD spectra of the pure lovastatin sample as per Fig.7, revealed sharp and intense peaks, indicating a highly crystalline structure. The presence of these distinct peaks is characteristic of the crystalline form of lovastatin, confirming its crystalline purity and quality.

The diffraction pattern of lovastatin showed prominent peaks at specific 2θ angles, which correspond to the known crystallographic planes of the drug. These sharp and intense peaks further verify the crystalline nature of the lovastatin sample used in this study, which is essential for its formulation into modified release tablets, as the crystalline form can influence the drug's solubility and bioavailability.

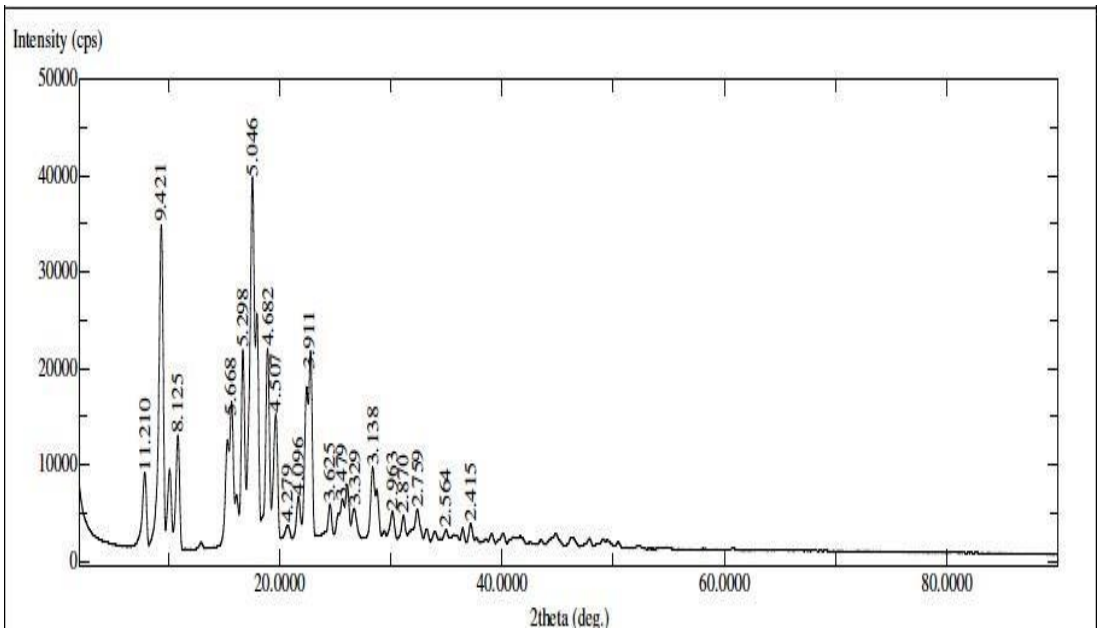

Fig. 7: XRD Study of Lovastatin

The diffraction pattern of oleanolic acid showed prominent peaks at specific 2θangles (Fig 8), which correspond to the known crystallographic planes of the drug. These sharp and intense peaks further verify the crystalline nature of the oleanolic acid sample usedin this study, which is essential for its formulation into modified release tablets, as the crystalline form can influence the drug's solubility and bioavailability.

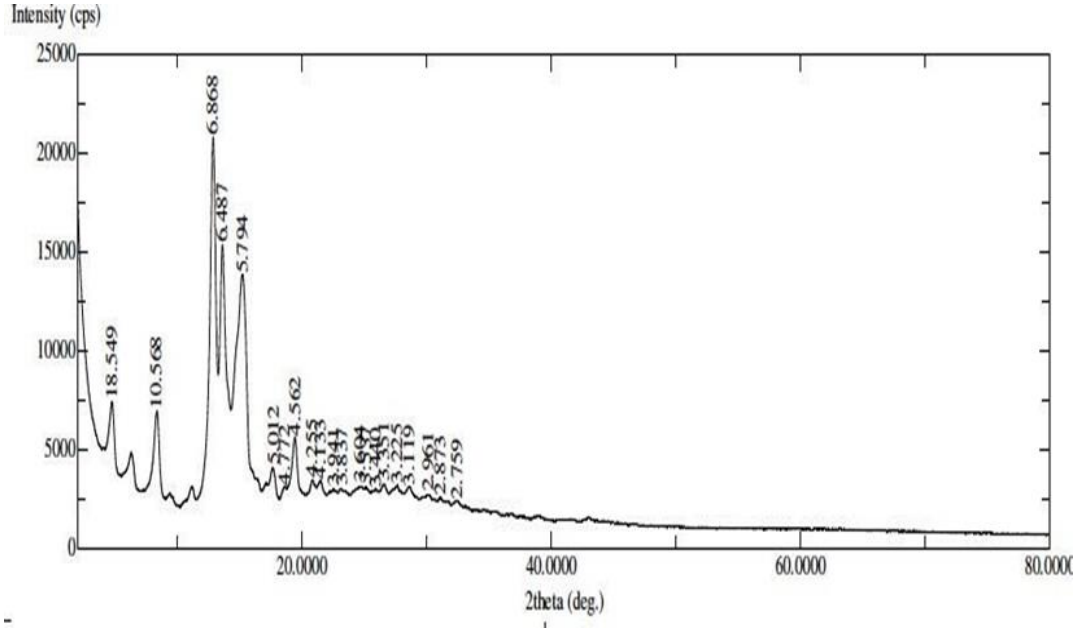

Fig. 8: XRD Study of Oleanolic Acid

7.5 Analytical method Development

To establish an effective technique for the simultaneous estimation of lovastatin and oleanolic acid, various mobile phases were evaluated to achieve optimal separationof the two drugs. The chromatograms of the same are summarized in Fig. 9,10 and 11. The following observations were made:

Initial Mobile Phase Trials: The system using acetonitrile (ACN) and methanol in a 50:50 (v/v) and 20:80 (v/v) ratios did not provide a satisfactory number of theoretical plates, indicating inadequate separation.

Optimized Mobile Phase: Subsequent trials with different compositions led to the selection of methanol (MeOH) and water adjusted to pH 3 with orthophosphoric acid (OPA) in a 90:10 (v/v) ratio. This mobile phase configuration provided a significant improvement in the number of theoretical plates and resulted in well-defined peaks forboth lovastatin and oleanolic acid.

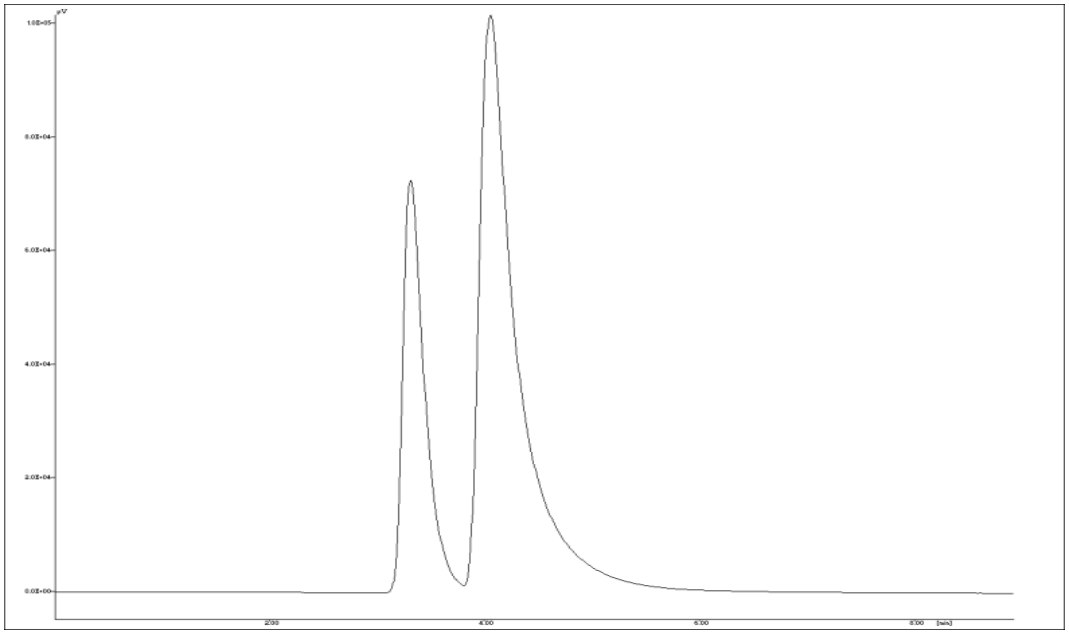

Fig. 9: Chromatogram of mixture of LV and OA with ACN: MEOH (50:50 v/v)

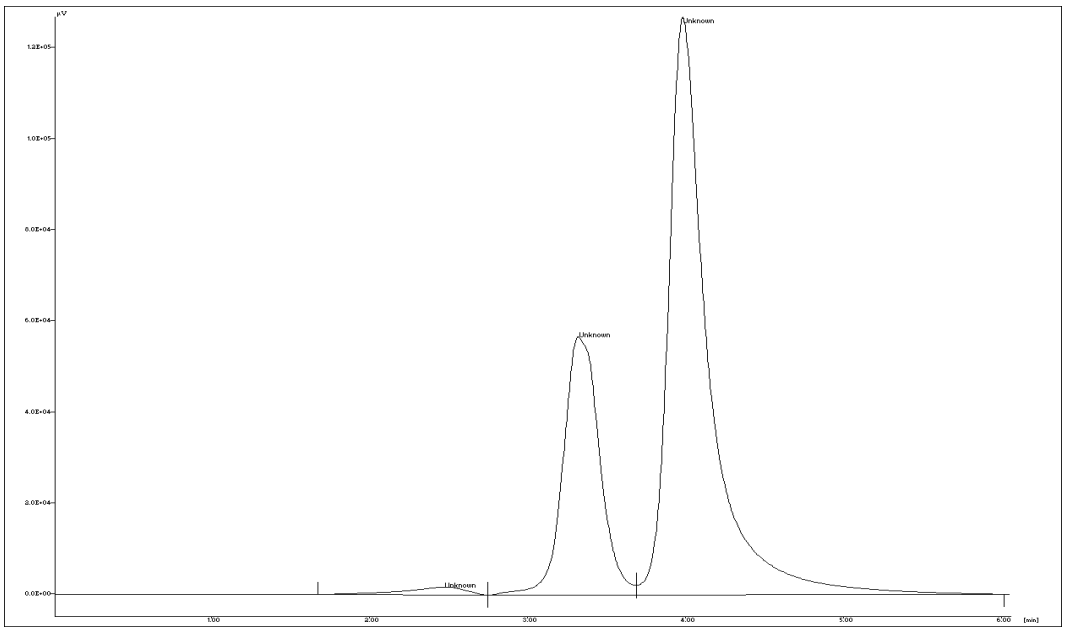

Fig. 10: Chromatogram of mixture of LV and OA with ACN:MEOH (20:80 v/v)

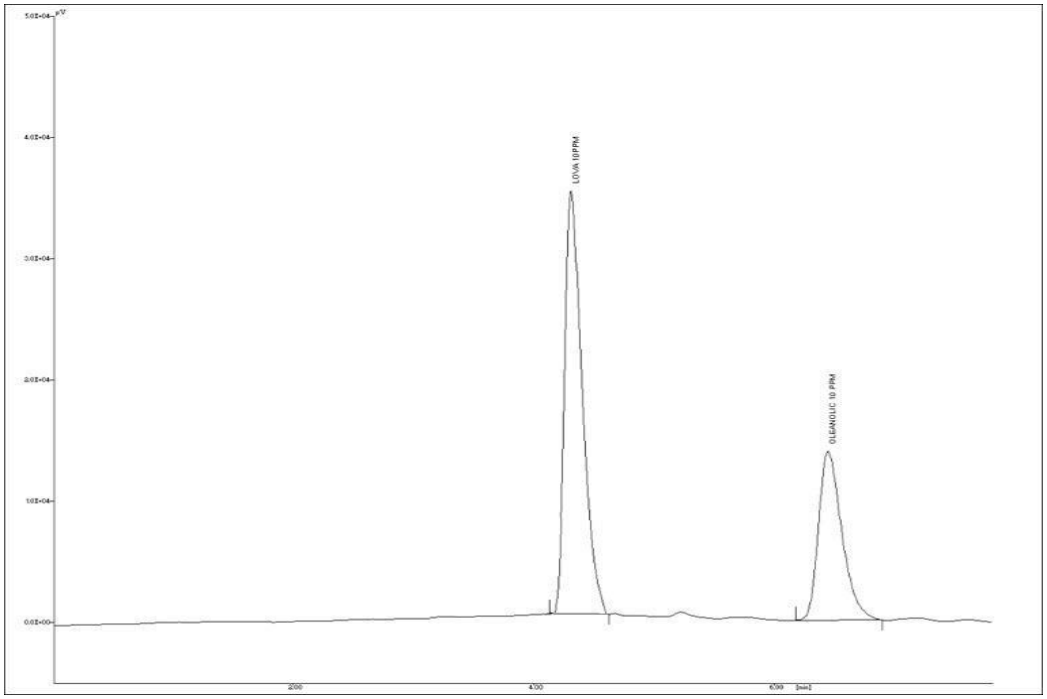

Fig. 11: Chromatogram of mixture of LV and OA with Methanol: Water pH 3 adjusted with OPA (90:10 v/v)

7.6 Technique validation

7.6.1 Preparation of quality control standards

The preparation involved appropriate dilution in the mobile phase to achieve final concentrations ranging from 2-12 µg/mL for both oleanolic acid and lovastatin. These working solutions were used for the chromatographic analysis and technique validation.

7.6.2 Selection of wavelength

To determine the optimal detection wavelength for simultaneous estimation, the standard stock solutions of oleanolic acid and lovastatin were further diluted with methanoland scanned over the wavelength range of 200-400 nm. The resulting spectra as shown in Fig. 12, 13 and 14, indicated that both drugs exhibited significant absorbance at 210 nm. This wavelength was selected for the detection of lovastatin and oleanolic acid during RP-HPLC analysis, ensuring accurate and sensitive quantification of both compounds.

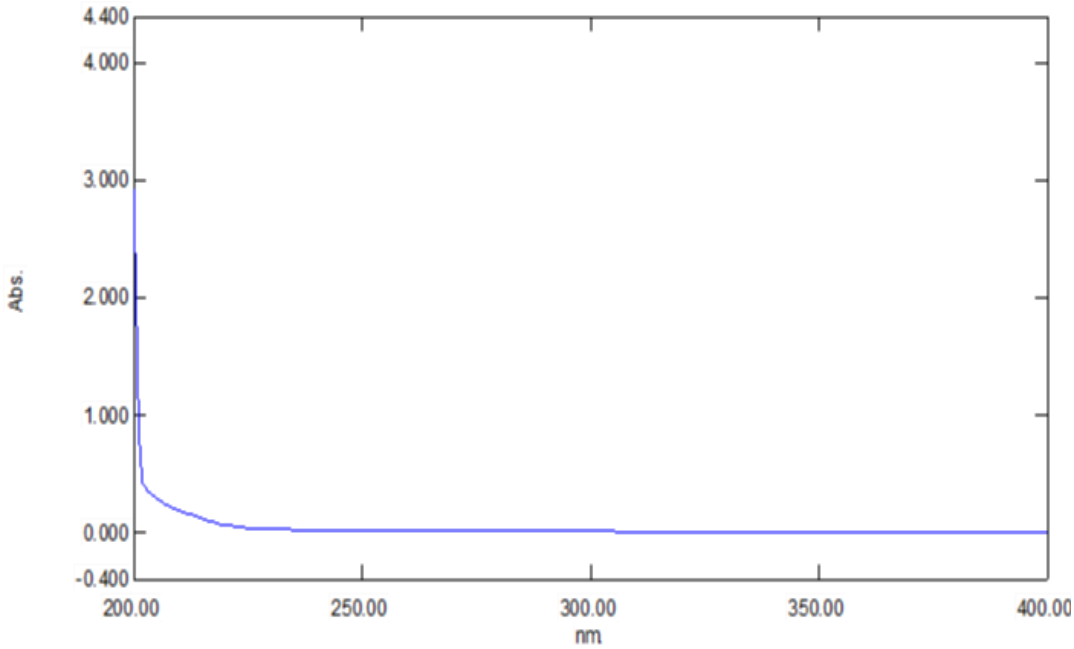

Fig. 12: UV-VIS Spectra of Oleanolic acid

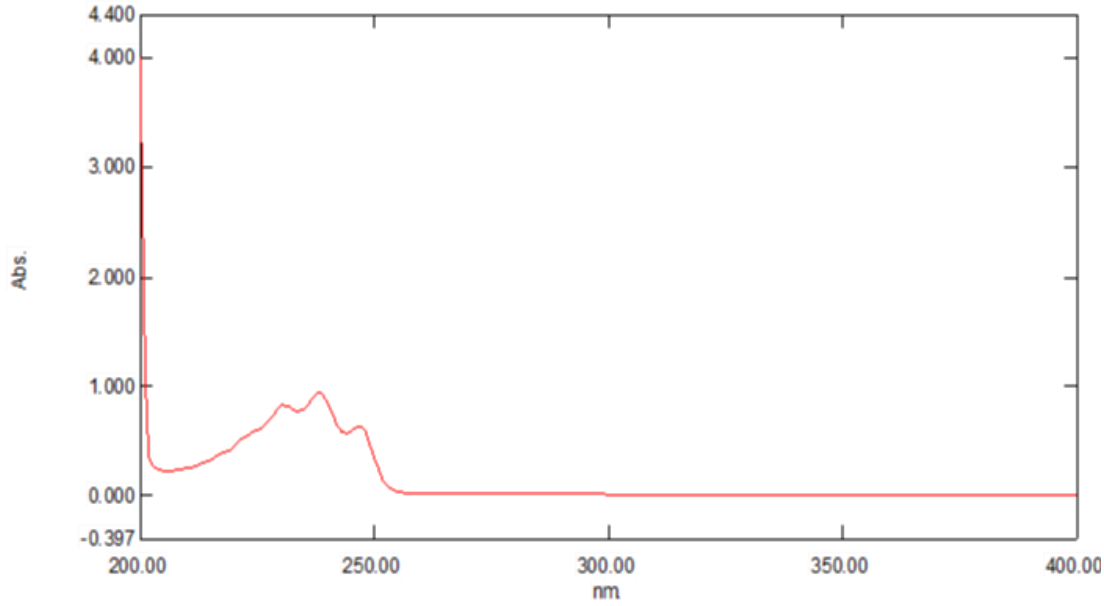

Fig. 13: UV-VIS Spectra Lovastatin

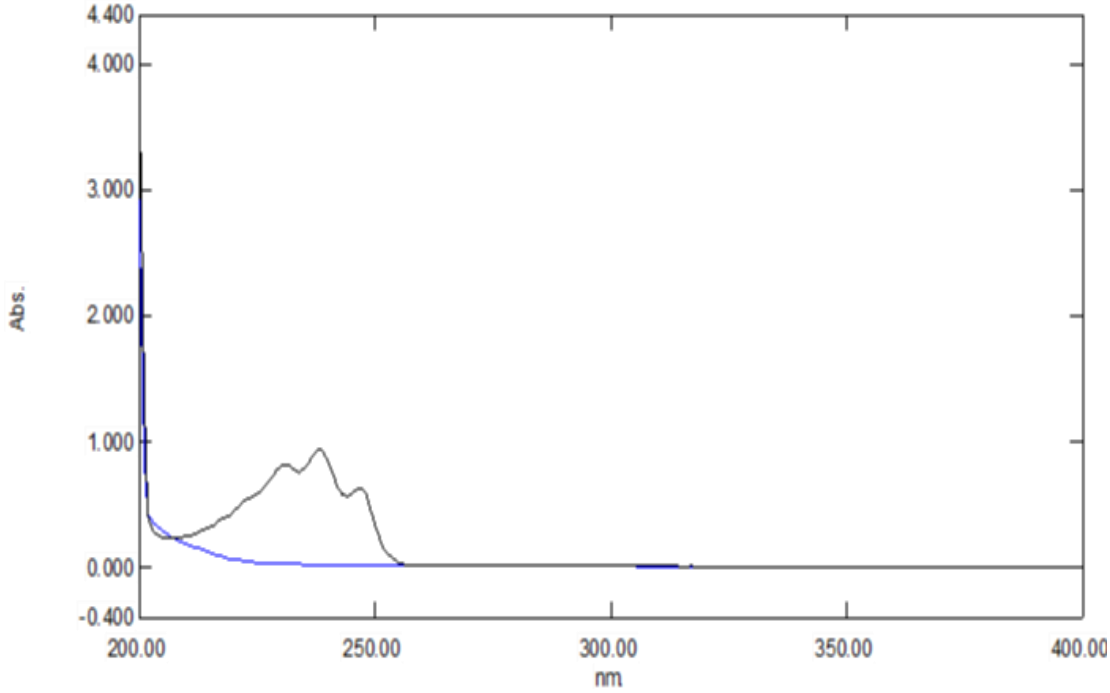

Fig. 14: Overlay Spectrum of OA and LV

7.6.3 Specificity

The specificity of the technique was determined by analyzing blank samples, standard solutions of lovastatin and oleanolic acid, and their mixture. The technique demonstrated clear separation of lovastatin and oleanolic acid with no interference from excipients or other components in the matrix.

7.6.4 System Suitability

System suitability tests were performed to ensure that the HPLC system was operating correctly. Parameters for example retention time, peak shape, and theoretical plates were evaluated. The technique met the required system suitability criteria, ensuring the reliability of the results (Table 13). The system suitability parameters for lovastatin and oleanolic acid were assessed to ensure the performance and reliability of the RP-HPLC technique.

Table 13: System Suitability Parameters

Drug	Conc. (µg/mL)	RT (min)	Area	Plates	Asymmetry	Resolution
Lovastatin	4	4.41	865950.68	2871.08	1.19	--
Oleanolic Acid	4	6.43	648344.75	2970.43	1.32	6.26

7.6.5 Linearity

Linearity was assessed by preparing standard solutions of lovastatin and oleanolic acid at various concentrations within the analytical range (2-12 µg/mL). The calibration curves were constructed (Fig. 15 and 16), and the linear regression analysis demonstrated a high correlation coefficient for both drugs, confirming the technique's ability to produce results that are directly proportional to the concentration of the analytes. The linearity of the RP-HPLC technique for oleanolic acid was evaluated by preparing solutions at different concentrations and measuring their peak areas. The linearity results of oleanolic acid and lovastatin are summarized in Table 14 and 15 respectively.

The linearity study demonstrates that the technique exhibits a strong linear relationship between the concentration of oleanolic acid and the corresponding peak areas across the tested range (2-12 µg/mL). The low % RSD values confirm the precision of the technique, making it appropriate for accurate quantification of oleanolic acid.

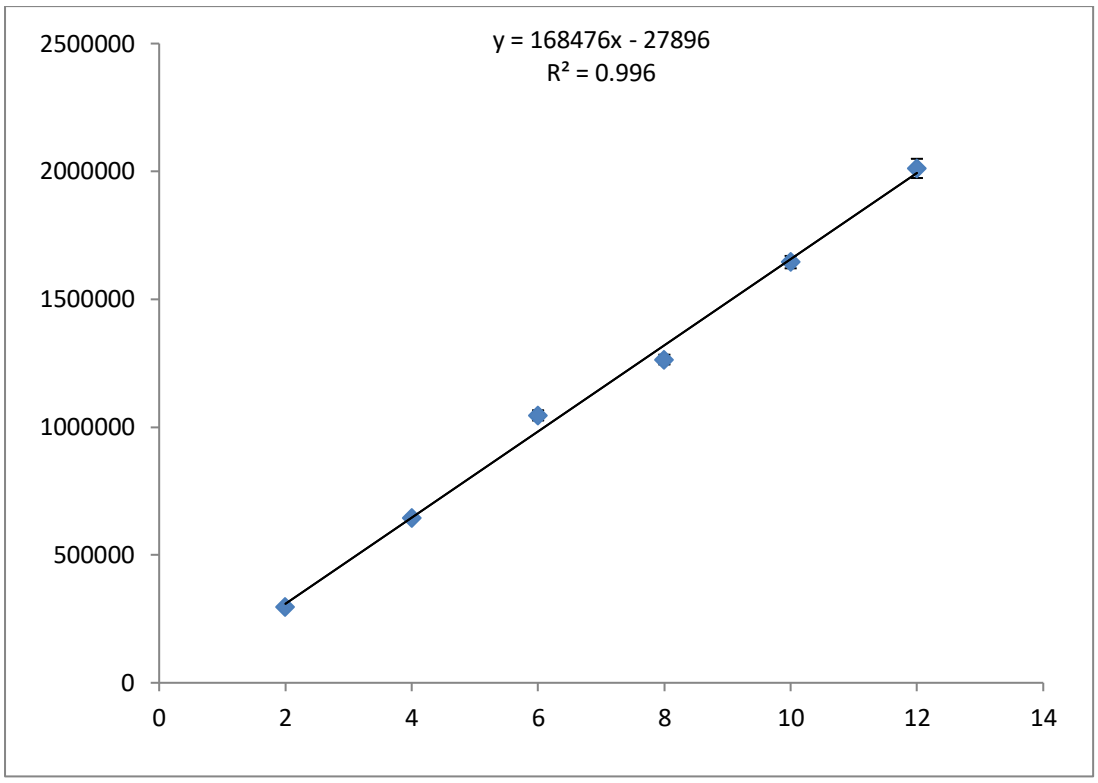

Fig. 15: Calibration Curve of Oleanolic acid

Table 14: Linearity Study of oleanolic acid

Conc. (µg/mL)	Area 1	Area 2	Area 3	Area 4	Area 5	Area 6	Average	SD	% RSD
2	297455.800	297757.760	297656.800	291964.900	297581.100	297667.880	296680.707	2312.472	0.779
4	648344.750	649876.660	642343.650	634563.660	633456.650	662321.980	645151.225	10808.379	1.675
6	1051637.300	1043454.760	1054538.000	1004245.650	1054325.800	1066893.980	1045849.248	21727.041	2.077
8	1262222.930	1287678.760	1276568.900	1235434.780	1243543.990	1278978.660	1264071.337	20883.337	1.652
10	1620619.860	1667876.770	1612345.700	1632147.500	1670543.660	1667493.700	1645171.198	26485.835	1.610
12	1996902.520	1965459.900	2076789.980	1995432.770	1990245.550	2045279.800	2011685.087	41100.695	2.043

- Average: The mean of the peak areas obtained for each concentration.
- SD (Standard Deviation): A measure of the dispersion of peak areas around the average.
- % RSD (Relative Standard Deviation): The percentage of the standard deviation relative to the average peak area, indicating the precisionof the technique.

The linearity of the RP-HPLC technique for lovastatin was evaluated by preparing solutions at different concentrations and measuring their peak areas. The results are summarized in Table 11. The linearity study indicates that the RP-HPLC technique provides a good linear relationship between the concentration of lovastatin and the corresponding peak areas across the tested range (2-12 µg/mL). The low % RSD values confirm the technique's precision and suitability for accurate quantification of lovastatin.

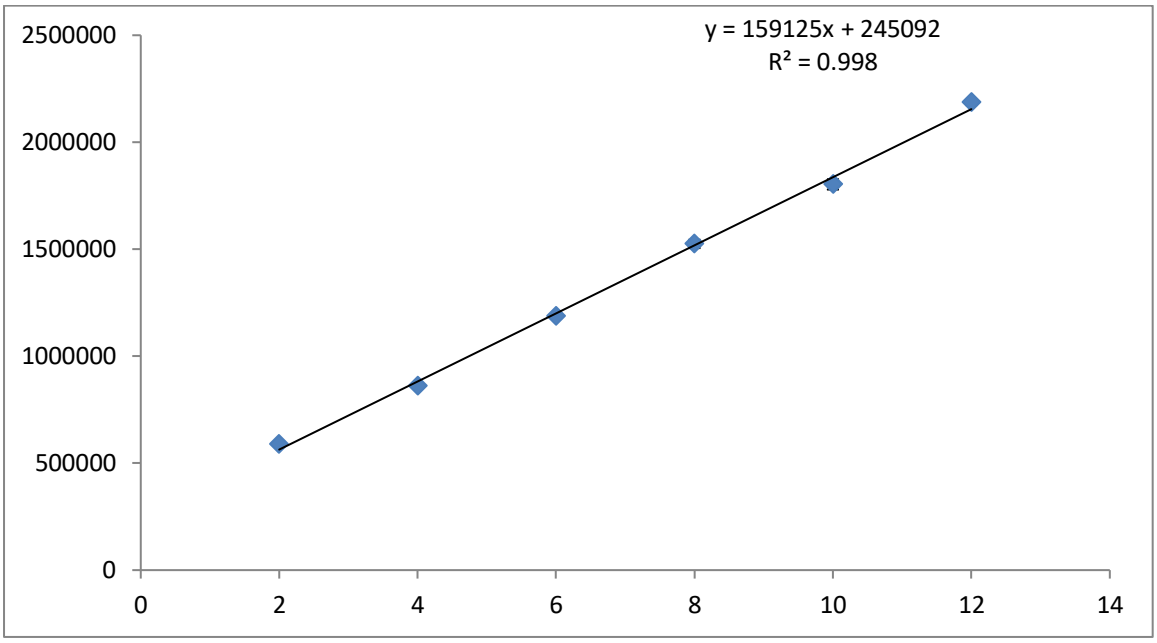

Fig. 16: Calibration Curve of Lovastatin

Table 15: Linearity Study of Lovastatin

Conc. (µg/mL)	Area 1	Area 2	Area 3	Area 4	Area 5	Area 6	Average	SD	% RSD
2	570769.190	595514.633	595630.330	599999.600	585489.967	592885.267	590048.165	10588.476	1.795
4	865950.680	858863.567	855251.627	866262.200	866965.933	858848.660	862023.778	4974.736	0.577
6	1194078.443	1158965.300	1189114.323	1198928.600	1195886.293	1192196.630	1188194.932	14698.399	1.237
8	1504230.820	1521911.487	1507804.290	1533262.257	1518125.663	1562566.260	1524650.130	21272.522	1.395
10	1787201.487	1795998.627	1792302.867	1798189.267	1787846.633	1855847.960	1802897.807	26300.771	1.459
12	2190175.963	2193188.293	2192783.267	2199659.633	2163225.267	2176854.923	2185981.224	13446.392	0.615

- Average: The mean of the peak areas obtained for each concentration.
- SD (Standard Deviation): A measure of the variability in peak areas.
- % RSD (Relative Standard Deviation): The percentage of the standard deviation relative to the average peak area, indicating the precisionof the technique.

7.6.6 Accuracy

Accuracy was evaluated by spiking known quantities of lovastatin and oleanolic acid into the matrix and analyzing the spiked samples. The percentage recovery of both drugs was determined, and the technique showed recovery rates within acceptable limits (typically 98-102%), confirming the accuracy of the technique. To check the accuracy of the technique, recovery studies were performed by adding standard drug to the sample at three different levels: 50%, 100%, and 150%. The basic concentrations of the sample chosen were 4 μg/mL of Oleanolic Acid and 4 μg/mL of Lovastatin. These solutions were injected into the HPLC system in triplicate to obtain chromatographs. The drug concentrations of Oleanolic acid and Lovastatin were calculated using their respective linearity equations. The results obtained are shown in Tables 16 and 17. The results of the recovery studies indicate that the developed technique is accurate for the estimation of Oleanolic acid and Lovastatin, with mean % recovery values close to 100% and low % RSD values.

Table 16: Recovery Studies of oleanolic acid

Level	Sample Conc. (µg/mL)	Std. Conc. (µg/mL)	Area	Concentration (µg/mL)	% Recovery	Mean % Recovery ± % RSD
50%	4	2	1038349.750	5.998	99.960	100.310 ± 0.693
			1037324.770	5.992	99.859	
			1049976.120	6.067	101.110	
100%	4	4	1368709.500	7.958	99.481	99.616 ± 0.140
			1370435.770	7.969	99.609	
			1372453.250	7.981	99.759	
150%	4	6	1719654.760	10.042	100.415	99.555 ± 0.759
			1700048.990	9.925	99.252	
			1695767.800	9.900	98.998	

Table 17: Recovery Studies of Lovastatin

Level	Sample Conc. (µg/mL)	Std. Conc. (µg/mL)	Area	Concentration (µg/mL)	% Recovery	Mean % Recovery ± % RSD
50%	4	2	1195769.230	5.963	99.386	100.089 ± 0.627
			1207308.550	6.036	100.592	
			1204389.620	6.017	100.287	
100%	4	4	1522347.800	8.012	100.146	99.988 ± 0.179
			1520790.990	8.002	100.023	
			1517860.670	7.983	99.794	
150%	4	6	1841758.900	10.015	100.152	99.918 ± 0.206
			1835587.870	9.976	99.765	
			1836759.450	9.984	99.838	

7.6.7 Precision

The technique's precision was evaluated through intra-day and inter-day variability studies. For intra-day analysis, three replicates of three different concentrations were tested within a single day, and the percentage relative standard deviation (RSD) was determined. In the inter-day study, the same concentrations were analyzed over three consecutive days, and the percentage RSD was calculated to assess consistency. The results obtained for Intra-day and Inter-day variations of Oleanolic acid are shown in Tables 18 and 19, and for Lovastatin in Tables 20 and 21 respectively. These results show that the technique exhibits good precision for Oleanolic acid and lovastatin with low % RSD values, indicating consistent and reliable measurements within the same day and over the different days.

Table 18: Intra-day Precision of oleanolic acid

Concentration (µg/mL)	Area	% Recovery	Avg % Recovery ± % RSD
4	650544.000	100.673	100.245 ± 0.551
	648976.670	100.441	
	643452.210	99.621	
6	996204.900	101.310	100.893 ± 0.528
	993819.190	101.074	
	985924.050	100.293	
8	1309467.940	99.225	99.994 ± 1.125
	1312790.240	99.472	
	1337234.890	101.285	

Table 19: Inter-day Precision of oleanolic acid

Concentration (µg/mL)	Area	% Recovery	Avg % Recovery ± % RSD
4	651349.070	100.793	100.740 ± 0.277
	648967.120	100.439	
	652677.830	100.990	
6	1000729.000	101.758	100.824 ± 1.070
	993782.120	101.071	
	979346.220	99.643	
8	1314562.360	99.603	99.745 ± 0.622
	1309238.890	99.208	
	1325621.440	100.424	

Table 20: Intra-day Precision Study of Lovastatin

Concentration (µg/mL)	Area	% Recovery	Avg % recovery ± % RSD
4	879284.6	99.450	100.478 ± 1.220
	894486.6	101.834	
	883744.7	100.149	
6	1207605.0	100.623	100.178 ± 0.890
	1193523.0	99.151	
	1208911.0	100.760	
8	1554631.0	102.677	101.569 ± 0.955
	1531639.0	100.874	
	1535243.0	101.157	

Table 21: Inter-day Precision Study of Lovastatin

Concentration (µg/mL)	Area	% Recovery	Avg % recovery ± % RSD
4	883086.010	100.046	99.937 ± 0.339
	879966.660	99.557	
	884115.960	100.208	
6	1218557.300	101.768	100.452 ± 1.135
	1199641.150	99.791	
	1199710.780	99.798	
8	1537564.220	101.339	100.578 ± 1.025
	1533129.960	100.991	
	1512898.710	99.405	

7.6.8 Robustness

Robustness was tested by deliberately varying the chromatographic conditions for example flow rate, mobile phase composition, and wavelength. In order to find out small and deliberate changes on robustness of the developed technique, the analysis was performed by making slight change in the ratio of flow rate (0.95,1,1.05 mL/min), mobile phase ratio of Methanol and Water (92:8, 90:10 and 88:12 v/v) and wavelength(209,201,211 nm). The standard solution was injected three times for each

varied conditions and its effect on peak area, recovery and retention time was examined and recorded. The robustness results of Lovastatin and Oleanolic acid are summarized in Table 22 and 23 respectively. The percentage relative deviation observed from the robustness study was found to be less than 2% for Lovastatin and Oleanolic acid which specifies that the proposed technique was satisfactorily robust.

Table 22: Robustness study for lovastatin

Variables	Value	Lovastatin				
		Conc. (µg/mL)	Mean peakArea (N=3)	SD	% RSD	Mean % RSD
Mobile phase ratio (A: B) v/v	92:08	4	888304.58	3489.50	0.39	0.57
	90:10	4	880998.20	4965.32	0.56	
	88:12	4	885857.78	6672.56	0.75	
Flow rate (mL/min)	0.95	4	884758.45	3475.56	0.39	0.44
	1.00	4	885040.65	2174.03	0.24	
	1.05	4	887311.65	6129.85	0.69	
Wavelength (nm)	209	4	883723.79	4890.54	0.55	0.55
	210	4	880002.10	4774.93	0.54	
	211	4	883707.82	5113.73	0.57	

Table 23: Robustness study for oleanolic acid

Variables	Value	Oleanolic acid				
		Conc. (µg/mL)	Mean peakArea (N=3)	SD	% RSD	Mean % RSD
Mobile phase Ratio (A: B)v/v	92:08	4	641062.177	2616.313	0.408	0.369
	90:10	4	642037.213	2352.470	0.366	
	88:12	4	640069.620	2125.532	0.332	
Flow rate(mL/min)	0.95	4	645340.287	7417.739	1.149	1.026
	1.00	4	643721.713	9795.305	1.522	
	1.05	4	643628.993	2626.785	0.408	
Wavelength (nm)	209	4	637847.787	2463.044	0.386	0.376
	210	4	644593.493	2573.058	0.399	
	211	4	646319.543	2211.619	0.342	

7.6.9 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The obtained result of LOD and LOQ indicates that the developed technique is sensitive for the detection and quantification of both Lovastatin and Oleanolic Acid at low concentrations. The validation results in Table 24 confirm that the developed RP-HPLC technique is reliable, accurate, andappropriate for the simultaneous estimation of lovastatin and oleanolic acid.

Table 24: LOD and LOQ of Lovastatin and Oleanolic Acid

Parameter	LV (µg/mL)	OA(µg/mL)
Limit of Detection	0.169	0.250
Limit of Quantification	0.512	0.758

7.7 Formulation of core and coat tablets

7.7.1 Preformulation study of Core Tablet Formulation

The preformulation study of granules was conducted to evaluate their physical properties, which are critical for the formulation of core tablets. The study included measurements of bulk density, tapped density, Hausner ratio, Carr's Index, and angle of repose. The results are summarized in Table 25.

Table 25: Preformulation study for Core Tablet Formulation

Batch No.	Bulk Density (g/mL)	Tapped Density (g/mL)	Hausner Ratio	Carr's Index (%)	Angle of Repose (Degrees)
OA1	0.53	0.45	1.09	9	16
OA2	0.53	0.45	1.11	11	18
OA3	0.52	0.42	1.13	13	20
OA4	0.49	0.43	1.11	11	17.50
OA5	0.48	0.41	1.11	11	18
OA6	0.52	0.47	1.21	21.50	29
OA7	0.45	0.51	1.42	13.33	21
OA8	0.44	0.49	1.39	22.50	28.40
OA9	0.46	0.49	1.39	28.94	32

7.7.1.1 Bulk Density

The bulk density of the granules ranged from 0.44 to 0.53 g/mL. Higher bulk densities (e.g., OA1, OA2) suggest better flow properties, while lower bulk densities (e.g., OA8, OA9) may affect the uniformity of tablet formation.

7.7.1.2 Tapped Density

Tapped densities ranged from 0.41 to 0.51 g/mL. The variation indicates differences in the granule's ability to compact, which is important for ensuring consistent tablet quality.

7.7.1.3 Hausner Ratio

The Hausner ratio values ranged from 1.09 to 1.42. A Hausner ratio less than 1.20 indicates good flow properties, which is ideal for tablet manufacturing. Batches like OA1, OA2, and OA4 fall within this desirable range. Higher ratios (e.g., OA7, OA8, OA9) suggest poorer flowability, which may necessitate formulation adjustments.

7.7.1.4 Carr's Index

Carr's Index values ranged from 9% to 28.94%. Values below 15% generally indicate good flowability and compressibility, which are beneficial for tablet production. Higher Carr's Index values (e.g., OA6, OA8, OA9) suggest that the granules have poorer flow properties and may require process optimization.

7.7.1.5 Angle of Repose

The angle of repose values ranged from 16 to 32 degrees. Lower angles (e.g., OA1, OA4) indicate better flow properties, while higher angles (e.g., OA8, OA9) suggest that the granules may have flow issues. Formulations with higher angles of repose may need the addition of flow aids or modifications in the processing conditions.

7.7.1.6 Hardness

All batches showed adequate hardness (Table 26), ranging from 3.8 to 5.0 kg/cm², which ensure that the tablets have good mechanical strength and are appropriate for handling and storage.

7.7.1.7 Friability

The friability of the tablets ranged from 0.18% to 0.24%, indicating that the tablets are resistant to mechanical abrasion and has acceptable durability.

Table 26: Evaluation of Core Tablets

Batch No	Hardness (kg/cm²)	Friability (%)
OA1	3.8	0.23
OA2	4.2	0.18
OA3	4.8	0.24
OA4	4.7	0.21
OA5	5.0	0.19
OA6	4.2	0.20
OA7	4.5	0.18
OA8	4.8	0.21
OA9	4.8	0.23

7.7.1.8 Dissolution Study of Core Tablets

The % drug release at different time points indicates variability among different batches, suggesting formulation-specific effects on the release profile. The dissolution profiles provide critical insights into the release characteristics of oleanolic acid from different formulations. This data is essential for optimizing the formulation for desired release characteristics and ensuring quality and efficacy of the final product (Table 27). As per the dissolution data shown in Fig 17, percentage drug release of oleanolic acid at 12h is more in OA1 formulation (97.13±0.961). So formulation OA1 is considered as optimized batch in in core tablet formulation.

The superior dissolution performance of formulation OA1 can be attributed to the optimal balance between hydrophilic HPMC K100 and hydrophobic ethyl cellulose. HPMC K100 promotes rapid hydration and gel layer formation, facilitating drug diffusion, while ethyl cellulose modulates the release by providing a diffusion barrier. This synergistic polymer interaction results in sustained yet complete drug release, making OA1 the most suitable core formulation.

The surface response plot for the release of oleanolic acid at 2 hours highlights the impact of varying concentrations of HPMC K100 and Ethyl Cellulose on drug release (Fig. 18). As Oleanolic Acid is water-insoluble, the presence of HPMC K100, a hydrophilic polymer, plays a essential role in the initial drug release.

The surface response plot for the release of oleanolic acid at 12 hours (Fig. 19) provides insight into the long-term release behavior of the tablets. The initial release

is primarily influenced by HPMC K100, which facilitates faster dissolution of the Oleanolic Acid. Ethyl Cellulose's impact is less significant at this stage. The high viscosity of HPMC K 100 creates a viscous layer that slows down the release rate over time. Ethyl Cellulose also contributes to the retardation of drug release, as it forms a semi-permeable membrane that restricts the drug's movement.

Table 27: Drug Release Profile of core tablets

Time (h)	OA1 (%)	OA2 (%)	OA3 (%)	OA4 (%)	OA5 (%)	OA6 (%)	OA7 (%)	OA8 (%)	OA9 (%)
1	16.54 ± 0.701	12.94 ±0.821	13.73 ± 0.830	22.66 ± 0.591	19.78 ± 0.849	14.12 ± 0.636	21.54 ± 0.630	18.96 ± 0.584	25.82 ± 0.904
2	22.54 ± 0.649	18.23 ± 1.036	19.72 ± 0.581	28.32 ± 0.575	23.43 ± 0.944	21.64 ± 0.802	27.32 ± 0.520	26.22 ± 0.380	32.23 ± 0.597
3	31.10 ± 0.492	25.85 ± 0.564	26.70 ± 0.655	36.55 ± 0.764	30.60 ± 0.564	29.25 ± 0.698	34.95 ± 0.558	32.96 ± 0.995	38.25 ± 0.750
4	49.50 ± 0.910	38.30 ± 0.792	42.44 ± 0.792	45.90 ± 0.610	41.63 ± 0.787	39.84 ± 0.434	43.55 ± 0.670	42.23 ± 0.422	42.44 ± 0.901
6	82.75 ± 0.807	72.66 ± 0.738	73.32 ± 0.957	79.34 ± 0.842	77.96 ± 0.556	75.55 ± 0.659	80.22 ± 0.609	73.88 ± 0.667	75.83 ± 0.299
8	91.65 ± 0.869	79.42 ± 0.422	81.55 ± 0.616	87.64 ± 0.808	86.35 ± 0.732	81.60 ± 0.581	85.25 ± 0.875	80.33 ± 0.936	79.23 ± 0.397
10	95.23 ± 0.946	83.47 ± 0.877	84.32 ± 0.666	88.41 ± 0.391	87.52 ± 0.563	82.62 ± 0.602	85.32 ± 0.564	81.27 ± 0.567	80.23 ± 0.781
12	97.13 ± 0.961	85.14 ± 1.087	86.01 ± 0.823	90.18 ± 0.528	89.27 ± 0.394	84.27 ± 0.821	87.03 ± 0.559	83.96 ± 0.505	82.83 ± 0.395

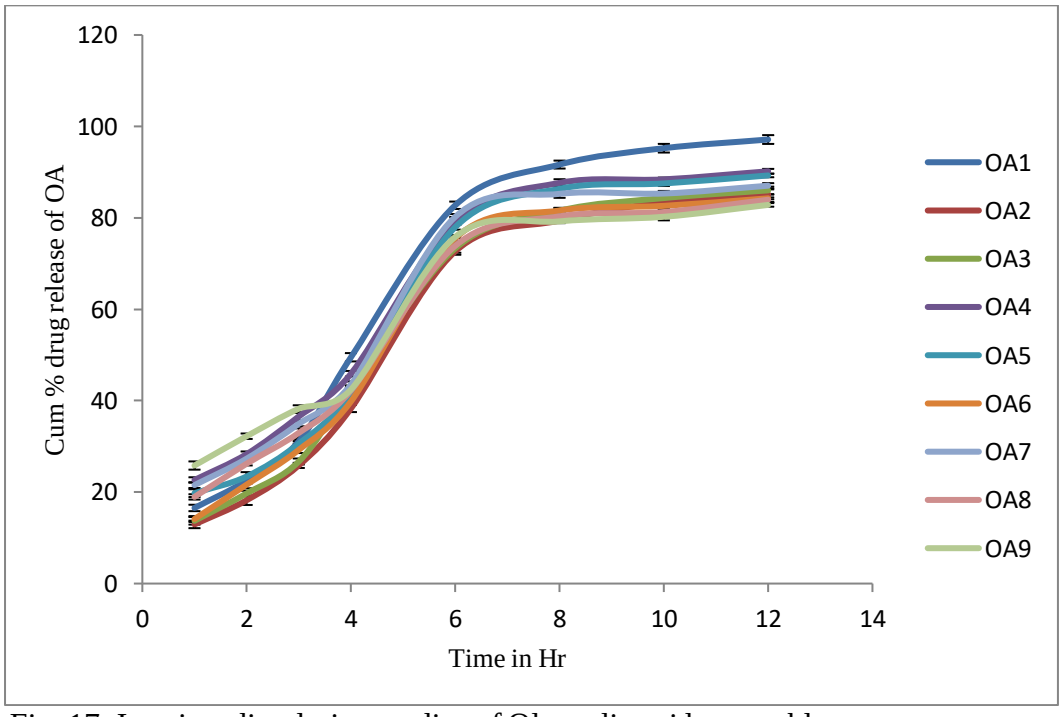

Fig. 17: In- vitro dissolution studies of Oleanolic acid core tablets

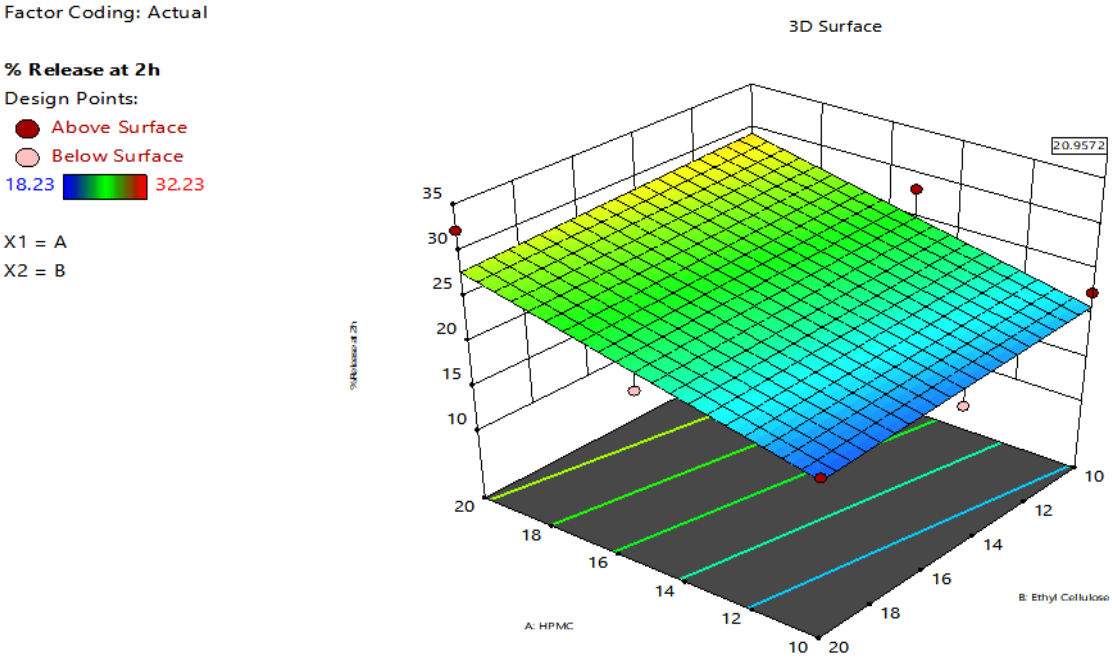

Fig. 18: Surface Response Plot of Core Tablet at 2 h

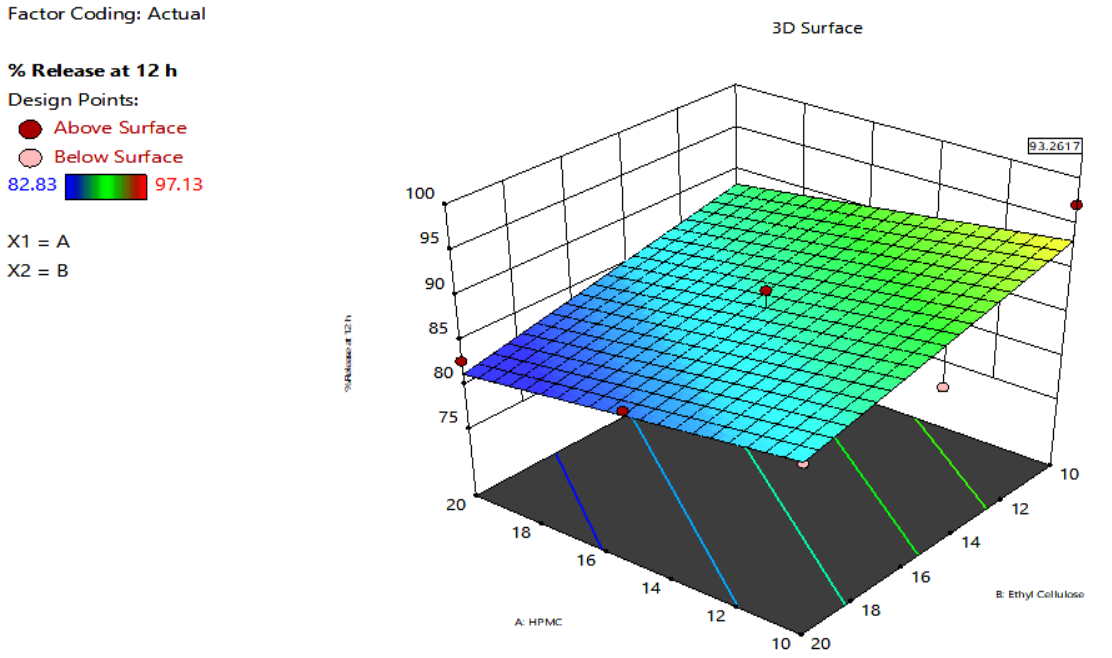

Fig. 19: Surface Response Plot of Core Tablet at 12 h

At early time points, HPMC K100 predominantly governs drug release due to its rapid swelling and hydration behavior. At later stages, the increased viscosity of the hydrated HPMC matrix along with the semi-permeable nature of ethyl cellulose contributes to diffusion-controlled release, thereby sustaining drug release over an extended period.

The similarity factor (f_2) study was carried out to compare the dissolution profile of the optimized formulation OA1 with the other developed formulations. The f_2 value is a logarithmic reciprocal square root transformation of the sum of squared differences between the test and reference dissolution profiles over all sampling time points. According to regulatory guidelines, f_2 values between 50 and 100 indicate similarity between two dissolution profiles, whereas values below 50 indicate dissimilarity. In the present investigation, the optimized formulation OA1 showed f_2 values less than 50 when compared with the other formulations, indicating that the drug release pattern of OA1 was significantly different from the remaining batches. This difference may be attributed to variations in the concentration of release-retarding polymers such as HPMC K100 and Ethyl Cellulose, which influenced the matrix integrity and drug diffusion characteristics. The distinct dissolution behavior of OA1 confirms its unique sustained-release profile and supports its selection as the optimized formulation for further studies.

7.7.2 Characterization of coating tablet

The preformulation study of coating blend was conducted to evaluate their physical properties. The study included measurements of bulk density, tapped density, Hausner ratio, Carr's Index, and angle of repose. The results are summarized in Table 28.

Table 28: Evaluation of coating powder blend

Batch No.	Bulk Density (g/mL)	Tapped Density(g/mL)	Hausner Ratio	Carr's Index (%)	Angle of Repose(Degrees)
LV 1	0.49	0.53	1.15	15.22	21.20
LV 2	0.61	0.71	1.18	18.33	26.30
LV 3	0.57	0.59	1.07	7.27	20.00
LV 4	0.51	0.68	1.13	13.33	21.30
LV 5	0.53	0.64	1.07	6.67	18.20
LV 6	0.54	0.67	1.12	11.67	24.70
LV 7	0.50	0.67	1.12	11.67	24.70
LV 8	0.48	0.54	1.08	8.00	21.40
LV 9	0.59	0.62	1.05	5.08	19.60

7.7.2.1 Bulk Density

The bulk density values range from 0.48 to 0.61 g/mL. Lower bulk density indicates a higher porosity of the blend, which can affect the flow properties.

7.7.2.2 Tapped Density

Tapped density ranges from 0.53 to 0.71 g/mL. Higher tapped density usually indicate better compressibility.

7.7.2.3 Hausner Ratio

The Hausner ratio ranges from 1.05 to 1.18. Values below 1.2 suggest good flowability, while higher values indicate poor flowability.

7.7.2.4 Carr's Index

Carr's Index ranges from 5.08% to 18.33%. Lower Carr's Index values (below 10%) indicate excellent flow properties, whereas higher values suggest poorer flowability.

7.7.2.5 Angle of Repose

The angle of repose ranges from 18.2 to 26.3 degrees. Lower angles suggest better flowability, while higher angles indicate poorer flow properties.

The evaluation of the coating powder blend suggests that most batches have acceptable flow and compressibility properties, with some variability. Batches with a Hausner Ratio below 1.2 and Carr's Index below 15% are considered to have good flowability,

which is critical for uniform coating during tablet processing.

7.7.2.6 Hardness

All batches showed (Table 29) adequate hardness, ranging from 4.2 to 5.5 kg/cm², which ensure that the tablets have good mechanical strength and are appropriate for handling and storage.

7.7.2.7 Friability:

The friability of the tablets ranged from 0.10% to 0.20%, indicating that the tablets are resistant to mechanical abrasion and has acceptable durability.

7.7.3 Dissolution study of coating blend tablet

The dissolution data (Table 30) indicates that the percentage drug release of lovastatin at 30 min is highest in the LV9 formulation, reaching 95.23±0.393%. In- vitro dissolution studies (Fig. 20) suggests that LV9 provides the most efficient and highest release of the drug over time. Because of its superior performance, LV9 is considered the optimized batch in the coating blend tablet.

In surface response plot of lovastatin at, both SSG and CCS, as superdisintegrants, significantly enhance drug release and reduce disintegration time. SSG, with its dual mechanism of swelling and wicking, demonstrated a slightly higher impact on drug release compared to CCS (Fig. 20 and 21). The enhanced dissolution performance of formulation LV9 is primarily due to the optimized concentration of superdisintegrants, which promotes rapid water uptake and tablet disintegration. Sodium starch glycolate, through its swelling action, and croscarmellose sodium, via wicking, collectively accelerate tablet breakup, resulting in faster and more efficient drug release.

Table 29: Evaluation of coating Tablets

Batch No	Hardness (kg/cm²)	Friability (%)
LV1	4.2	0.20
LV2	4.6	0.12
LV3	5.1	0.16
LV4	5.3	0.14
LV5	4.8	0.16
LV6	4.5	0.15
LV7	4.9	0.13
LV8	5.3	0.14
LV9	5.5	0.10

Table 30: Drug Release Profile of coating tablets

	Time (Min)							
	10	20	30	60	90	120	150	180
LV1	15.25± 0.633	32.4± 0.560	52.85± 0.831	70.2± 0.873	90.88± 0.758	98.1± 0.327	100.3± 0.609	99.9± 0.530
LV2	21.1± 0.509	42.65± 0.600	67.23± 0.484	88.2± 0.892	96.33± 0.520	100.65± 0.344	101.15± 0.714	99.96± 0.372
LV3	22.2± 0.746	47.95± 0.568	73.5± 0.735	94.2± 0.497	99.15± 0.679	100.36± 0.702	99.8± 0.821	100.25± 0.508
LV4	21.66± 0.572	44.8± 0.615	65.13± 0.555	95.65± 0.790	98.9± 0.556	100.1± 0.892	100.55± 0.805	101.1± 0.494
LV5	20.55± 0.321	49.53± 0.494	67.89± 0.687	94.3± 0.937	99.1± 0.890	100.25± 0.404	100.65± 0.834	100.1± 0.906
LV6	22.7± 0.490	46.1± 0.450	72.56± 0.640	95.2± 0.494	99.25± 0.703	99.98± 0.375	101.25± 0.487	100.9± 0.737
LV7	23.5± 0.589	51.25± 0.478	74.62± 0.553	96.23± 0.498	99.5± 0.633	100.46± 0.625	101.1± 0.365	100.8± 0.636
LV8	28.9± 0.592	52.75± 0.704	84.28± 0.475	97.23± 0.420	99.9± 0.484	100.15± 0.485	99.9± 0.749	100.45± 0.594
LV9	32.4± 0.425	63.7± 0.429	95.23± 0.393	96.23± 0.943	98.99± 0.638	100.8± 0.869	101.15± 0.360	100.5± 0.778

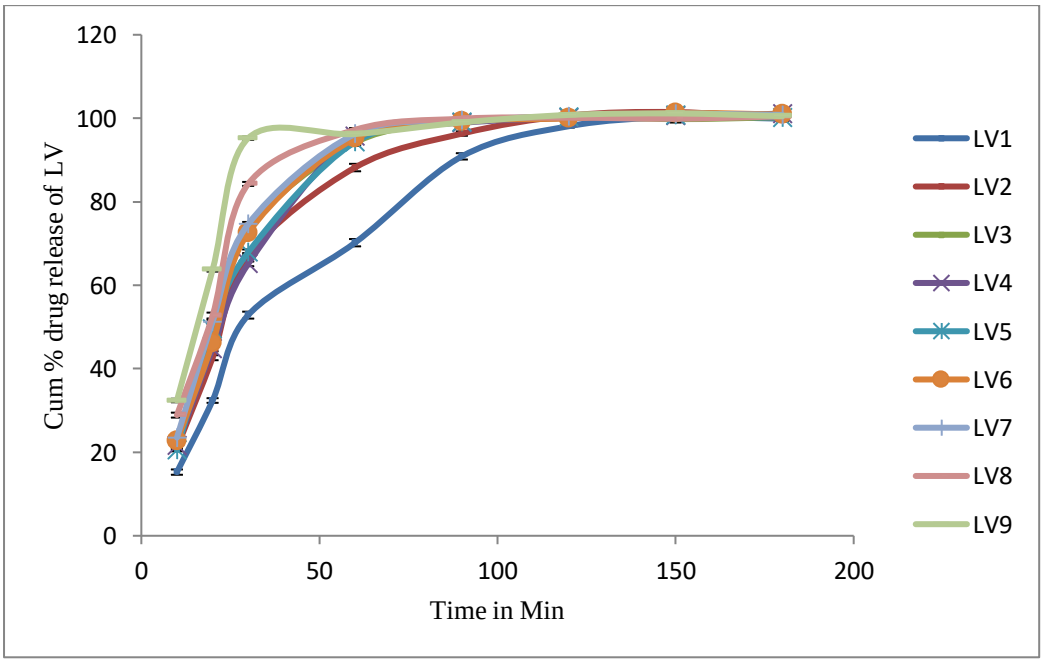

Fig. 20: In- vitro dissolution studies of lovastatin coating tablets

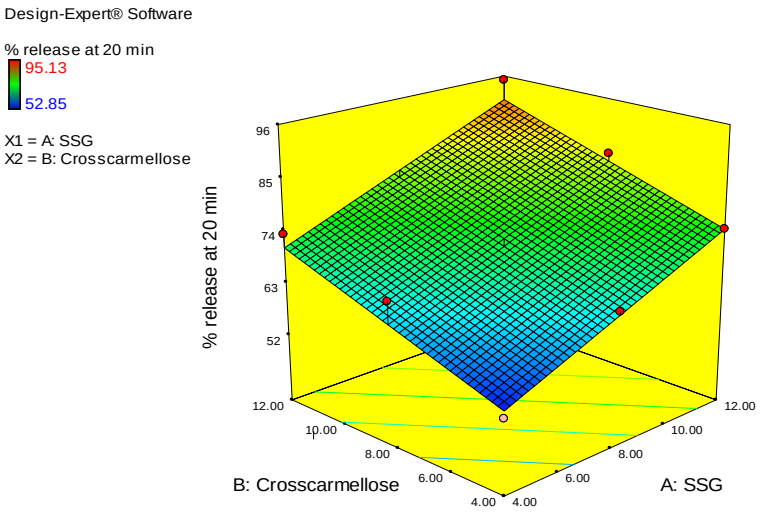

Fig. 21: Surface Response Plot of coating Tablet

7.7.4 The disintegration test (DT) of coat layer

The optimization process shown in Table 31, determined that Batch LV9 had a disintegration time of just 24 seconds. This rapid disintegration suggests a highly efficient drug release profile (Fig. 22), ensuring quick onset of action. Such a fast breakdown enhances bioavailability and improves therapeutic effectiveness. Given these advantages, Batch LV9 is considered the optimal choice for immediate release formulations.

The significantly lower disintegration time observed for LV9 can be attributed to the synergistic action of superdisintegrants, which enhances capillary action and swelling pressure, leading to rapid tablet breakup and immediate drug availability.

Table 31: Optimization of Lovastatin Immediate Release Coat

Batch	% Release of Lovastatin after 30 min	DT of Coat (sec)
LV1	52.85±0.831	98
LV2	67.23±0.484	75
LV3	73.5±0.735	72
LV4	65.13±0.555	78
LV5	67.89±0.687	72
LV6	72.56±0.640	56
LV7	74.62±0.553	62
LV8	84.28±0.475	36
LV9	95.23±0.393	24

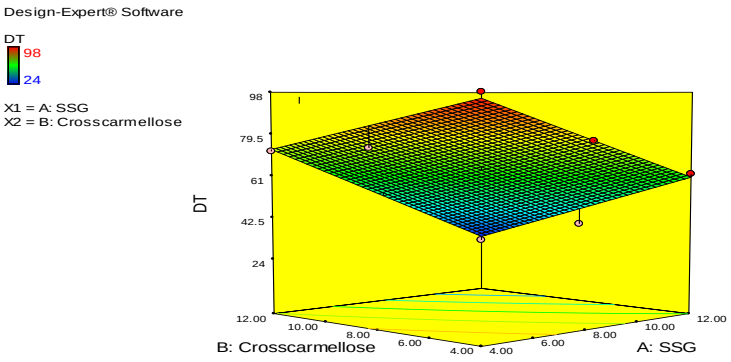

Fig. 22: Surface response plot of coating tablet DT.

7.7.5 Evaluation of core and coat tablets

The prepared core and coat tablet, consisting of the OA1 core tablet and LV9 coating blend, exhibited adequate hardness of 5.4 kg/cm². This ensures that the tablets possess good mechanical strength, preventing breakage during handling and storage.

The friability of the core and coat tablet was observed to be 0.20%, demonstrating excellent resistance to mechanical abrasion. This low friability ensures that the tablets remain intact during handling and storage.

7.7.6 Dissolution study of core and coat Tablets

The dissolution study demonstrates a controlled and gradual release of Oleanolic acid from the core and coat tablets (Table 32), achieving a high cumulative drug release by the end of the study period (Fig. 23). At 12 h, the formulation shows a drug release of 96.34%, indicating its effectiveness in sustaining drug release over time. This release profile confirms the efficiency of the optimized core and coat formulation in ensuring extended drug release.

The combined release behavior confirms the successful integration of sustained-release and immediate-release layers within a single dosage form. The core–coat design ensures prolonged release of oleanolic acid while enabling rapid availability of lovastatin, thereby fulfilling the intended therapeutic objective of combination therapy.

The results of the dissolution study (Table 33) indicate that the LV9 formulation ensures a rapid and near-complete release of Lovastatin. By 30 minutes, the formulation achieves approximately 91.24% drug release (Fig. 24), highlighting its efficiency in immediate release applications. This fast dissolution profile enhances the drug's bioavailability and ensures a quicker onset of action. The optimized formulation effectively meets the requirements for immediate release tablets.

Table 32: Dissolution study of OA from core and coat tablet

Time (h)	Cum % drug release of Oleanolic acid				Std Dev.
	I	II	II	Avg	
1	13.48	16.67	15.39	15.18	1.31
2	18.78	20.95	19.79	19.84	0.88
3	27.33	29.64	28.38	28.45	0.94
4	38.87	40.51	39.84	39.74	0.67
6	64.23	66.39	65.61	65.41	0.89
8	83.1	85.55	84.31	84.32	1.00
10	91.66	93.78	93.32	92.82	0.91
12	97.18	96.12	95.72	96.34	0.61

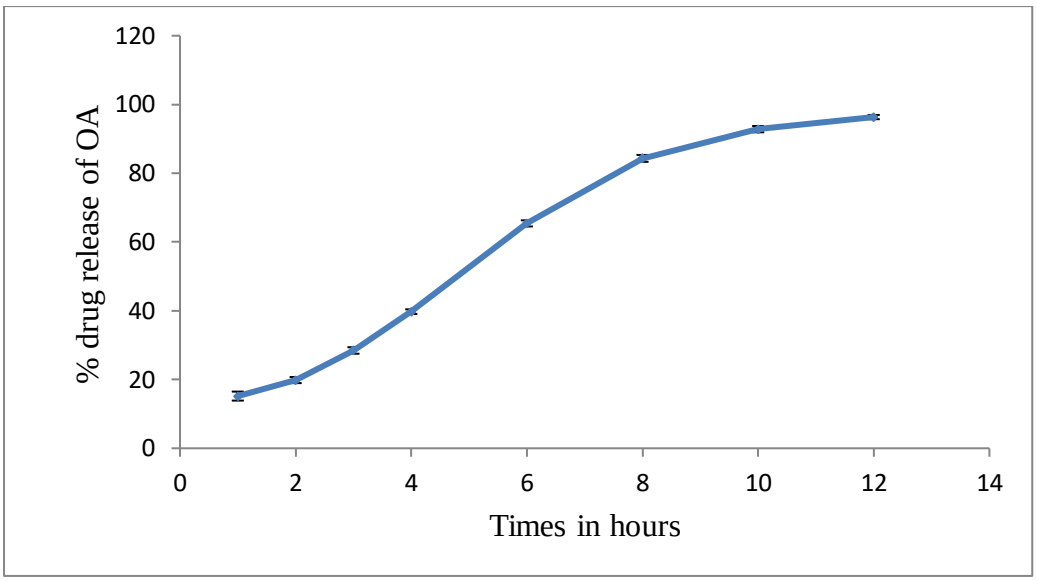

Fig. 23: Drug release of OA from core and coat tablet

Table 33: Dissolution study of LV from core and coat tablet

Time (min)	Cum % drug release of Lovastatin				Std Dev.
	I	II	II	Avg	
10	30.53	31.61	33.14	31.76	1.07
20	60.11	61.37	62.21	61.23	0.86
30	90.07	92.31	91.34	91.24	0.91
60	96.17	97.22	98.09	97.16	0.78
90	97.61	99.89	99.08	98.86	0.94
120	101.11	99.91	99.40	100.14	0.71
150	101.73	99.95	100.87	100.85	0.72
180	99.10	101.35	100.06	100.17	0.92

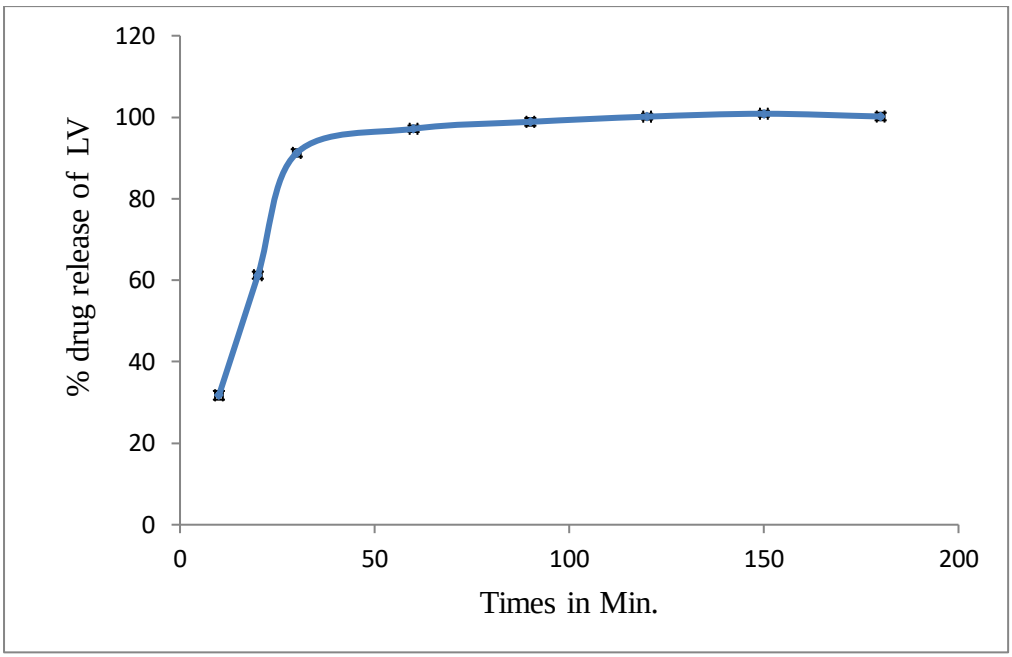

Fig. 24: Drug release of LV from core and coat tablet

7.8 Formulation of Bilayer tablets

7.8.1 Preformulation study of SR layer of bilayer

The preformulation study of granules was conducted to evaluate their physical properties, which are critical for the formulation of SR layer. The study included measurements of bulk density, tapped density, Hausner ratio, Carr's Index, and angle of repose. The results are summarized in Table 34.

Table 34: Preformulation study for SR Tablet Formulation

Batch No.	Bulk Density (g/mL)	Tapped Density (g/mL)	Hausner Ratio	Carr's Index (%)	Angle of Repose
BOA1	0.58	0.64	1.10	9.38	18.40
BOA2	0.60	0.63	1.05	4.76	18.60
BOA3	0.59	0.67	1.14	11.94	22.60
BOA4	0.50	0.63	1.26	20.63	14.50
BOA5	0.53	0.59	1.11	10.17	19.20
BOA6	0.56	0.61	1.09	8.20	24.30
BOA7	0.50	0.59	1.18	15.25	28.40
BOA8	0.47	0.58	1.23	18.97	29.40
BOA9	0.49	0.59	1.20	16.95	27.60

7.8.1.1 Bulk Density

The bulk density of the granules ranged from 0.47 to 0.60 g/mL. Higher bulk densities (BOA2) suggest better flow properties, while lower bulk densities (BOA8) may affect the uniformity of tablet formation.

7.8.1.2 Tapped Density

Tapped densities ranged from 0.58 to 0.67 g/mL. The variation indicates differences in the granule's ability to compact, which is important for ensuring consistent tablet quality.

7.8.1.3 Hausner Ratio

The Hausner ratio values ranged from 1.05 to 1.26. A Hausner ratio less than 1.20 indicates good flow properties, which is ideal for tablet manufacturing.

7.8.1.4 Carr's Index

Carr's Index values ranged from 4.76% to 20.63%. Values below 15% generally indicate good flowability and compressibility, which are beneficial for tablet production.

7.8.1.5 Angle of Repose

The angle of repose values ranged from 14.5 to 29.4 degrees. Lower angles indicate better flow properties, while higher angles suggest that the granules may have flow issues.

7.8.1.6 Hardness

All batches showed adequate hardness (Table 35), ranging from 4.52 to 5.42 kg/cm², which ensure that the tablets have good mechanical strength and are appropriate for handling and storage.

7.8.1.7 Friability

The friability of the tablets ranged from 0.20% to 0.26%, indicating that the tablets are resistant to mechanical abrasion and has acceptable durability.

Table 35: Evaluation of SR layer of bilayer tablets

Sr. No.	Batch No	Hardness	Friability
1	BOA1	5.40	0.24
2	BOA2	4.54	0.21
3	BOA3	5.08	0.26
4	BOA4	5.18	0.23
5	BOA5	5.42	0.21
6	BOA6	4.52	0.22
7	BOA7	4.76	0.20
8	BOA8	5.18	0.23
9	BOA9	5.19	0.25

7.8.2 Dissolution of SR layer of bilayer tablets

According to the dissolution data shown in Table 36, the BOA1 formulation exhibits the highest percentage drug release of oleanolic acid at 12 hours, reaching 98.18±0.647 %. This indicates superior drug release efficiency compared to other formulations (Fig. 25). As a result, BOA1 is considered the optimized batch for SR layer of bilayer tablet formulation. Its sustained release profile ensures better therapeutic efficacy and prolonged drug action.

The surface response plot for the release of OA SR layer at 2 hours shows (Fig. 26) an increase in HPMC and Ethyl Cellulose appears **to** modulate the drug release at 2 hours**.** Higher concentrations of HPMC & Ethyl Cellulose tend to reduce the initial release**,** leading to a more controlled release pattern.

The surface response plot for the release of OA SR layer at 12 hours provides insight into the long-term release behavior of the tablets (Fig. 27). The formulation effectively controls and sustains drug release**,** achieving 98.18% release at 12 hours**,** which is desirable for extended-release formulations. The nearly linear surface suggests that the drug release is predictable and consistent across the studied range of HPMC and Ethyl Cellulose concentrations. The optimized formulation ensures a gradual and prolonged release**,** making it appropriate for sustained drug delivery applications**.**

The sustained release behavior of oleanolic acid from the SR layer can be attributed to the combined effect of hydrophilic HPMC and hydrophobic ethyl cellulose. Upon contact with dissolution medium, HPMC hydrates and forms a gel layer, which acts as a diffusion barrier, while ethyl cellulose restricts rapid penetration of the medium. The

optimized formulation BOA1 exhibited a balanced polymer ratio, resulting in controlled drug diffusion and minimal initial burst release. This polymeric interaction explains the near zero-order release profile observed over 12 hours.

Table 36: Drug Release Profile of SR layer of bilayer tablets

Batch	Time (Hr)							
	1	2	3	4	6	8	10	12
BOA1	18.1± 0.483	20.65± 0.632	33.81± 0.486	51.95± 0.694	84.1± 0.348	90.95± 0.476	96.25± 0.347	98.18± 0.647
BOA2	14.3± 0.742	30.56± 0.34	35.15± 0.684	40.25± 0.69	74.36± 0.971	81.6± 0.617	90.12± 0.435	92.92± 0.483
BOA3	15.68± 0.473	23.25± 0.483	28.4± 0.243	44.62± 0.476	74.9± 0.314	83.1± 0.462	84.65± 0.846	86.34± 0.586
BOA4	18.6± 0.476	26.2± 0.852	38.85± 0.671	47.95± 0.317	78.2± 0.913	83.25± 0.648	84.48± 0.347	86.17± 0.436
BOA5	21.15± 0.713	28.85± 0.348	31.98± 0.674	43.5± 0.846	79.24± 0.694	81.2± 0.423	83.65± 0.348	85.32± 0.942
BOA6	16.35± 0.48	19.36± 0.348	31.65± 0.761	41.94± 0.348	77.45± 0.762	82.3± 0.348	84.38± 0.942	86.07± 0.348
BOA7	14.1± 0.743	18.4± 0.648	36.25± 0.348	45.2± 0.493	79.1± 0.347	81.25± 0.614	83.3± 0.431	85.97± 0.348
BOA8	18.64± 0.473	24.3± 0.672	34.68± 0.482	44.15± 0.347	75.8± 0.62	82.6± 0.347	88.18± 0.954	89.94± 0.672
BOA9	20.64± 0.469	33.65± 0.469	40.36± 0.647	44.85± 0.598	76.96± 0.347	79.4± 0.435	81.69± 0.397	84.32± 0.498

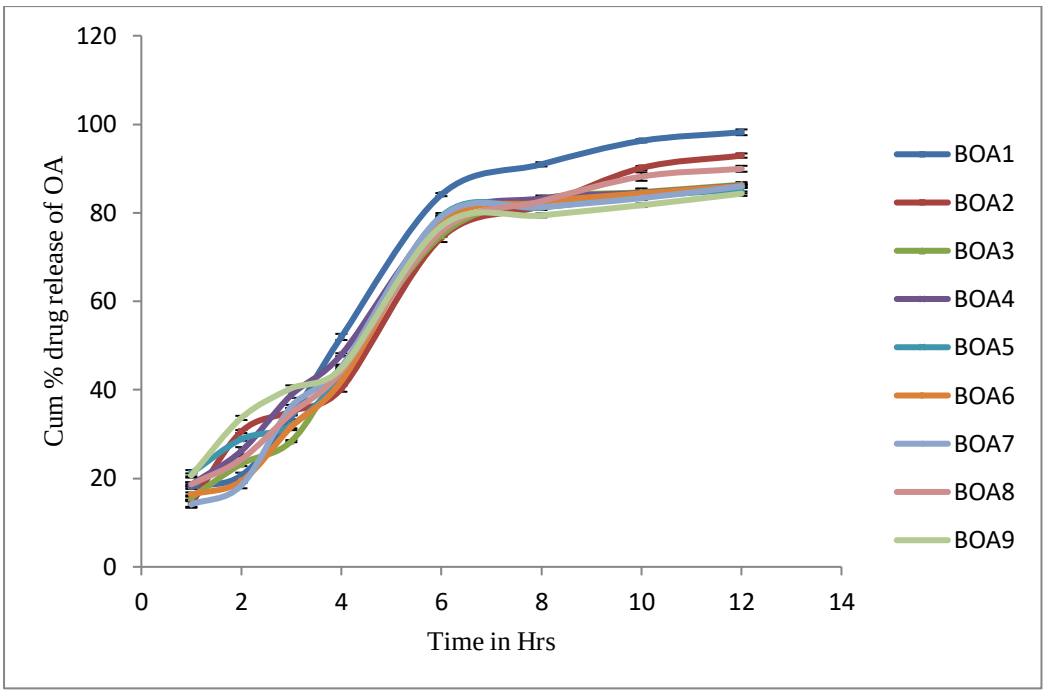

Fig. 25: In- vitro dissolution studies of SR layer of bilayer tablets

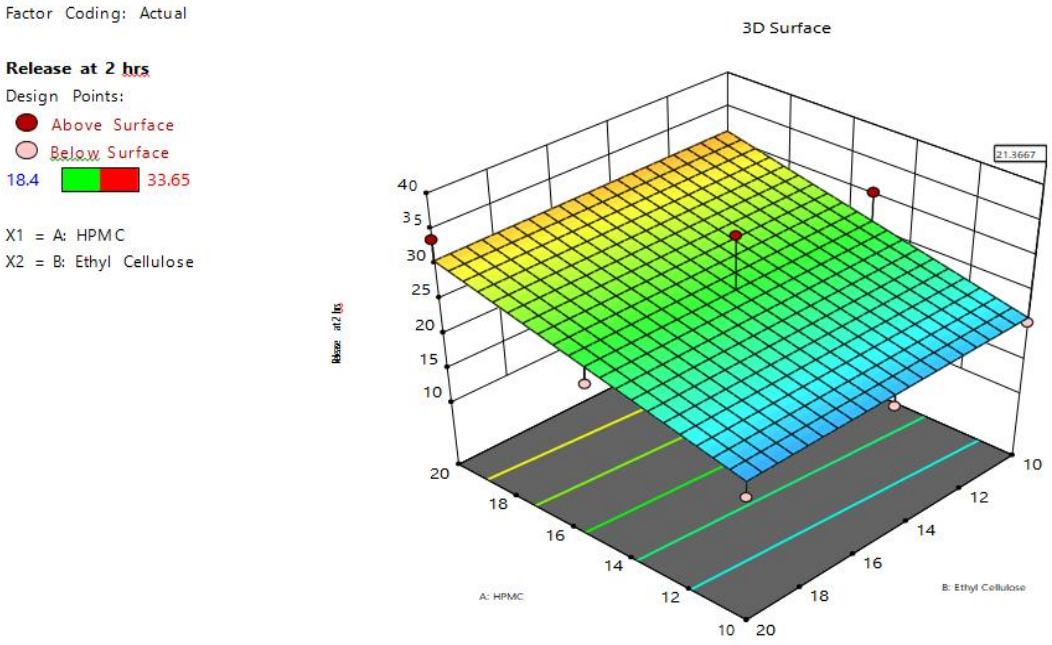

Fig. 26: Surface Response Plot of SR layer of bilayer tablets at 2 h

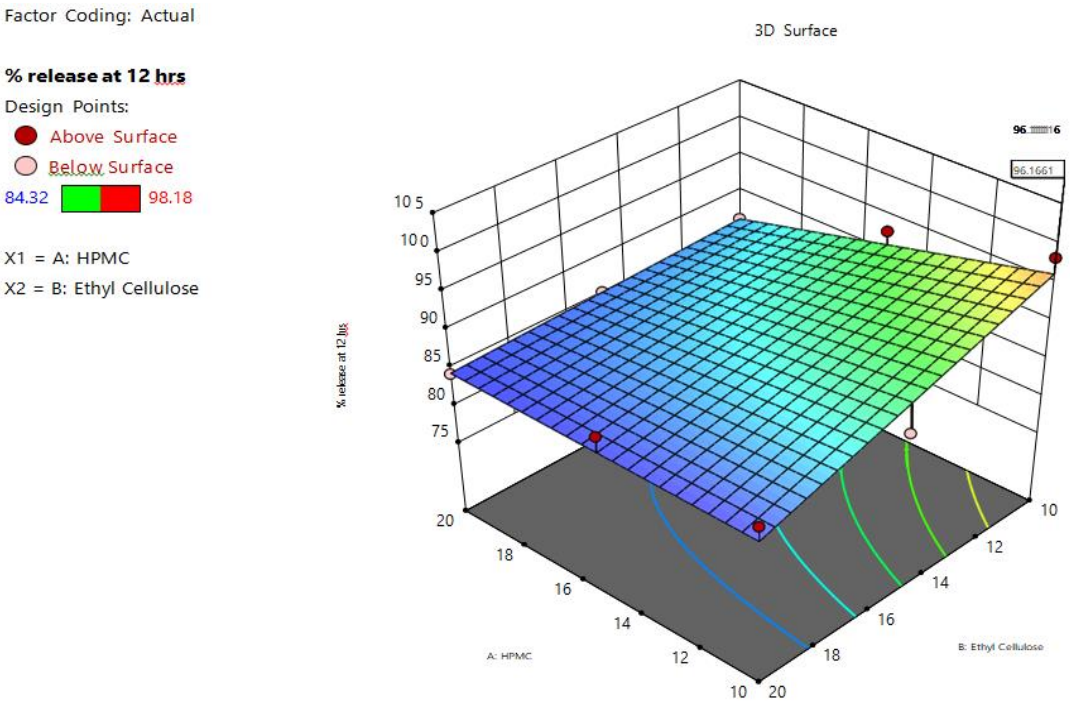

Fig. 27: Surface Response Plot of SR layer of bilayer tablets at 12 h

The sustained release behavior observed with higher HPMC K100 and ethyl cellulose concentrations is likely due to the formation of a dense gel barrier, which controls drug diffusion and matrix erosion over time.

7.8.3 Characterization IR layer of bilayer tablets

The preformulation study of the IR layer was conducted to assess its physical properties, including bulk density, tapped density, Hausner ratio, Carr's Index, and angle of repose. The findings of this evaluation are presented in Table 37.

Table 37: Evaluation of IR layer of bilayer tablets

Batch No.	Bulk Density (gm/mL)	Tapped Density (gm/mL)	Hausner Ratio	CarrIndex (%)	Angle of Repose (°)
BLV1	0.48	0.62	1.29	22.58	19.60
BLV2	0.63	0.72	1.14	12.50	24.30
BLV3	0.59	0.71	1.20	16.90	21.20
BLV4	0.48	0.61	1.27	21.31	21.30
BLV5	0.51	0.61	1.20	16.39	18.80
BLV6	0.52	0.61	1.17	14.75	23.80
BLV7	0.47	0.62	1.32	24.19	24.50
BLV8	0.45	0.56	1.24	19.64	23.40
BLV9	0.61	0.68	1.11	10.29	23.40

7.8.3.1 Bulk Density

The bulk density of the IR layer ranged from 0.45 to 0.63 g/mL, reflecting differences in powder compactibility and flow properties. A higher bulk density suggests better packing efficiency, while a lower value indicates a more porous structure.

7.8.3.2 Tapped Density

The tapped density of the IR layer ranged from 0.56 to 0.72 g/mL, indicating the maximum packing density of the powder. Higher tapped density values suggest better particle rearrangement under compression, leading to improved flow and packing efficiency.

7.8.3.3 Hausner Ratio

The Hausner ratio of the IR layer ranged from 1.11 to 1.32, providing insight into the powder's flow characteristics. Values above 1.25 indicate poor flowability, which may lead to challenges in uniform blending and tablet compression.

7.8.3.4 Carr Index

The Carr Index of the IR layer ranged from 10.29% to 24.19%, reflecting variations in powder flowability. Higher values indicate poorer flow properties, which may affect blending and compression during tablet formulation. Conversely, lower values suggest better flow characteristics, contributing to improved processing efficiency.

7.8.3.5 Angle of Repose

The angle of repose for the IR layer ranged from 18.8° to 24.5°, indicating variations in powder flowability. Higher angles suggest poorer flow properties, which may impact handling and processing efficiency.

7.8.3.6 Hardness

The hardness of the bilayer tablets summarized in Table 38, ranges from 4.7 to 5.7 Kg/cm^2. Higher hardness values are generally desirable as they indicate better mechanical strength and resistance to breakage during handling.

7.8.3.7 Friability

The friability values ranged from 0.14% to 0.28%, indicating the tablets' resistance to breakage and crumbling. Lower friability suggests better mechanical strength and durability, ensuring the tablets remain intact during handling and transportation.

Table 38: Evaluation of IR layer of bilayer tablets

Batch No.	Hardness (Kg/cm^2)	Friability (%)
BLV1	4.7	0.28
BLV2	4.8	0.16
BLV3	5.2	0.17
BLV4	5.5	0.16
BLV5	5.0	0.14
BLV6	4.8	0.18
BLV7	5.1	0.14
BLV8	5.5	0.15
BLV9	5.7	0.14

7.8.4 Dissolution of IR layer of bilayer tablets

The dissolution data reveals that the LV9 formulation achieved the highest percentage of drug release for lovastatin at 30 minutes (Table 39), reaching 95.23±0.943%. This indicates that LV9 provides the most efficient and rapid drug release compared to other formulations. The enhanced dissolution profile of LV9 suggests improved bioavailability (Fig. 28), which is essential for therapeutic efficacy. The superior performance of LV9 can be attributed to its optimized formulation and excipient selection, ensuring better solubility and dispersion. Given these advantages, LV9 is identified as the most effective formulation among the tested batches. Therefore, it is considered the optimized batch for the IR layer of the bilayer tablet.

The surface response graph (generated using Design-Expert® Software**)** of IR layer bilayer tablets indicates that both SSG and CCS significantly influence drug release at 30 minutes (Fig. 29). Higher concentrations of both excipients result in an increased release percentage, with the optimal release. The nearly symmetrical surface suggests that both factors contribute comparably to the response. Therefore, to achieve maximum drug release, an optimized combination of SSG and CCS should be used at higher levels.

The surface response graph of IR layer DT as per Fig. 30 shows that SSG and CCS significantly influence tablet disintegration time. Higher concentrations of these excipients lead to faster disintegration. The non-linear response suggests that both excipients work synergistically to enhance disintegration. Therefore, an optimal

formulation should include higher concentrations of SSG and CCS to achieve rapid tablet disintegration.

The rapid drug release observed in the IR layer formulations is mainly governed by the swelling and wicking mechanisms of superdisintegrants. Sodium starch glycolate and croscarmellose sodium rapidly absorb water, leading to tablet swelling and quick disintegration. Batch BLV9 demonstrated the shortest disintegration time and highest drug release at 30 minutes, indicating a synergistic effect of both superdisintegrants at optimized concentrations. This rapid disintegration enhances surface area exposure, resulting in improved dissolution and bioavailability of lovastatin.

Table 39: Drug Release Profile of IR layer of bilayer tablets

Batch	Time (Min)							
	10	20	30	60	90	120	150	180
BLV1	18.25± 0.498	35.55± 0.346	52.85± 0.625	72.20± 0.69	91.65± 0.483	99.10± 0.348	101± 0.756	99.80± 0.345
BLV2	23.50± 0.483	45.30± 0.698	67.23± 0.756	90.55± 0.364	98.60± 0.486	100.20± 0.684	99.80± 0.348	101.10± 0.598
BLV3	25.35± 0.648	50.55± 0.486	73.50± 0.985	95.20± 0.745	99.85± 0.358	99.90± 0.648	100.80± 0.368	99.90± 0.483
BLV4	22.66± 0.483	46.80± 0.681	65.13± 0.318	86.20± 0.478	96.65± 0.684	99.42± 0.792	100.25± 0.348	99.95± 0.982
BLV5	23.55± 0.483	47.33± 0.698	67.89± 0.348	91.35± 0.483	98.85± 0.467	100.10± 0.467	99.90± 0.843	101.35± 0.958
BLV6	24.65± 0.489	48.20± 0.364	72.56± 0.589	96.20± 0.317	99.85± 0.613	100.25± 0.314	99.15± 0.248	100.54± 0.347
BLV7	25.60± 0.483	50.65± 0.624	74.62± 0.792	99.44± 0.678	100.36± 0.468	99.85± 0.391	100.25± 0.34	99.98± 0.463
BLV8	30.90± 0.483	55.65± 0.324	84.28± 0.486	98.65± 0.308	100.65± 0.468	99.80± 0.716	101.25± 0.368	100.55± 0.409
BLV9	35.50± 0.483	65.70± 0.373	95.23± 0.943	99.68± 0.686	100.24± 0.761	100.60± 0.491	99.75± 0.678	100.85± 0.348

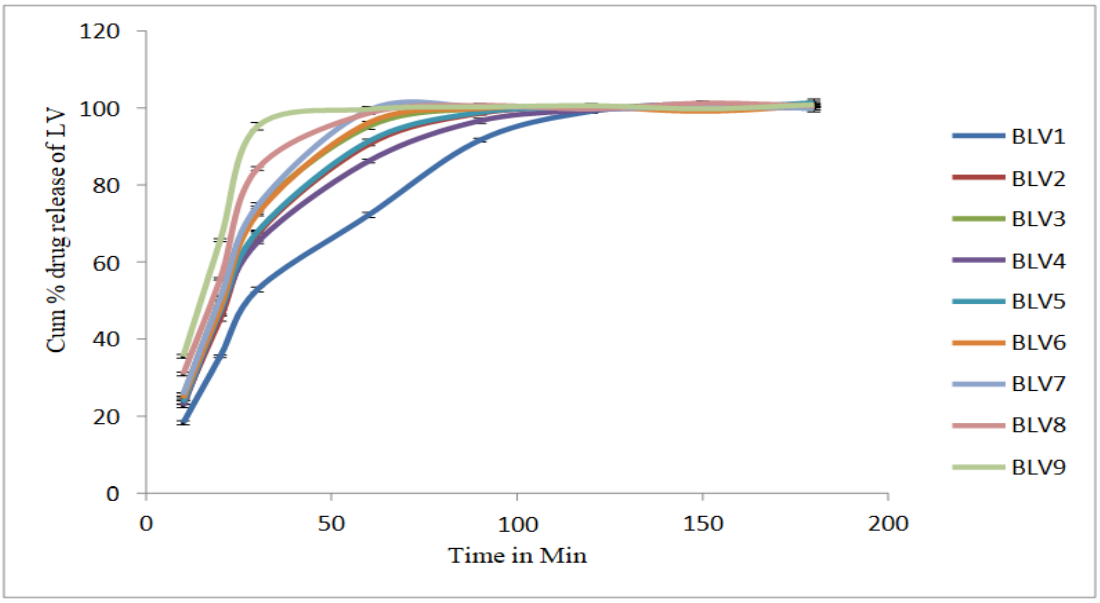

Fig. 28: In- vitro dissolution studies of IR layer of bilayer tablets

Statistical analysis and factorial design evaluation

The ANOVA analysis of the factorial design demonstrated that the independent variables X_1 and X_2 significantly influenced the drug release responses and disintegration time ($p < 0.05$). For Y_1 (% drug release at 2 h), significant effects were observed for X_1 ($F = 18.42$, $p = 0.003$), X_2 ($F = 14.25$, $p = 0.005$), and their interaction term X_1X_2 ($F = 5.12$, $p = 0.041$). Similarly, for Y_2 (% drug release at 12 h), X_1 ($F = 22.16$, $p = 0.002$), X_2 ($F = 25.41$, $p = 0.001$), and X_1X_2 ($F = 8.64$, $p = 0.019$) showed significant effects. The high F-values and low p-values indicated that sodium starch glycolate (SSG) and croscarmellose sodium (CCS) significantly affected the responses. The lack of fit was non-significant ($p > 0.05$), confirming model adequacy. Furthermore, the close agreement between R^2, adjusted R^2, and predicted R^2 values indicated good model fitness and predictability, while adequate precision values greater than 4 confirmed the reliability of the developed models.

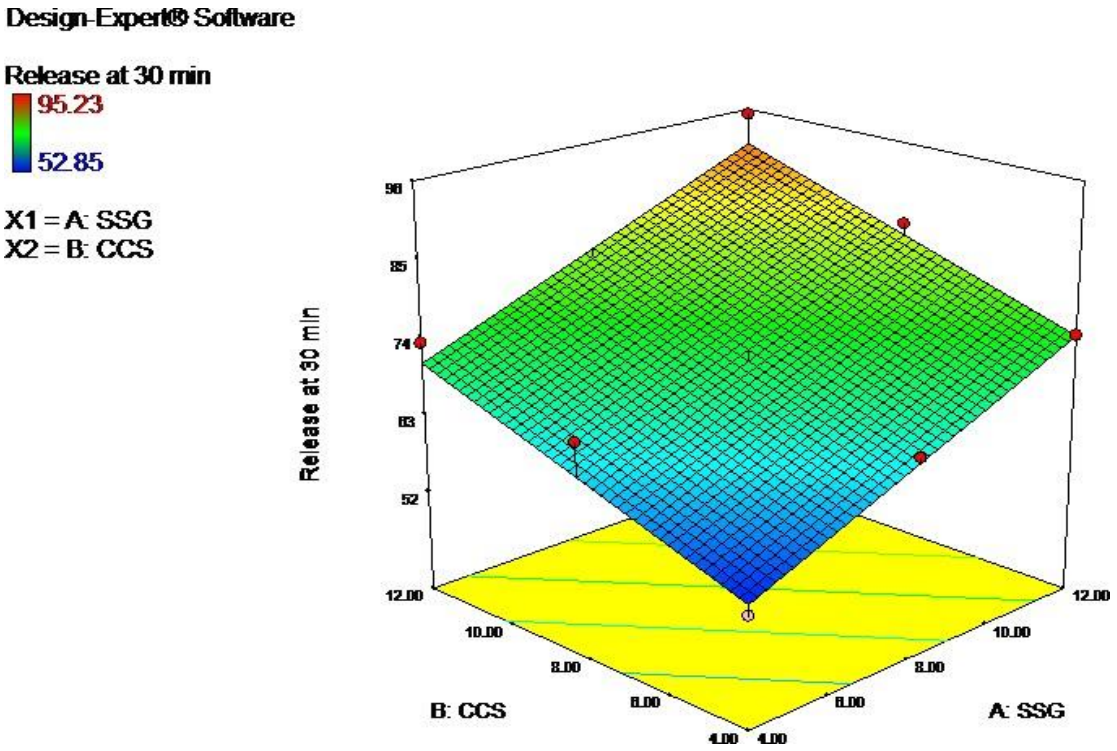

Fig. 29: Surface Response Plot of IR layer

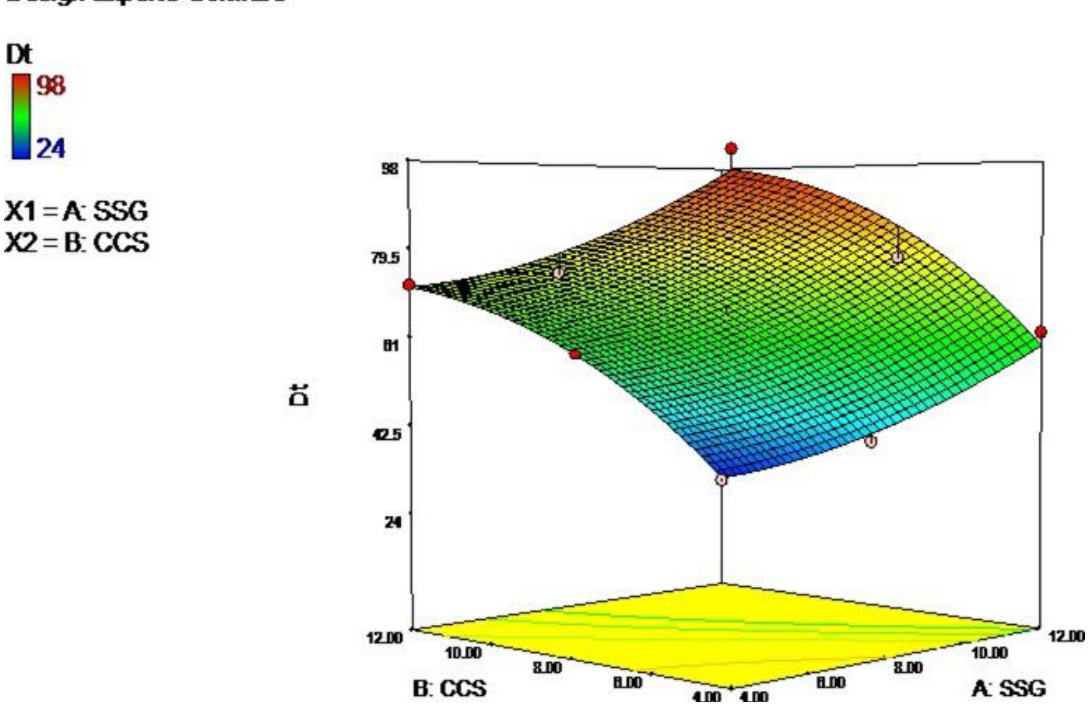

Fig. 30: Surface Response Plot of IR layer DT

The response surface plot for % drug release at 30 minutes (Fig. 29) shows that increasing concentrations of both SSG and CCS significantly enhanced lovastatin release. The nearly symmetrical surface indicates a comparable contribution of both

factors. The positive interaction suggests that simultaneous optimization of both superdisintegrants is required for maximum drug release. The response surface plot for disintegration time (Fig. 30) indicates a significant reduction in disintegration time with increasing levels of SSG and CCS. The curvature of the plot confirms a synergistic interaction between the two excipients, resulting in faster tablet disintegration.

The performance of the developed bilayer tablets can be explained by the mechanistic roles of formulation excipients and release-controlling polymers. In the IR layer, sodium starch glycolate and croscarmellose sodium promote rapid drug release through combined swelling and wicking mechanisms, leading to fast tablet disintegration and increased surface area for dissolution. The factorial design confirmed a synergistic interaction between these superdisintegrants, which was reflected in the statistically significant ANOVA results and response surface analysis.

7.8.5 The disintegration test (DT) of IR layer of bilayer tablets

Batch BLV9 exhibited a remarkably fast disintegration time of 24 seconds (Table 40), indicating an efficient drug release profile. This rapid breakdown facilitates quicker absorption, leading to enhanced bioavailability and improved therapeutic outcomes. Such characteristics make it well-suited for immediate-release formulations, ensuring a swift onset of action. Considering these benefits, Batch BLV9 stands out as the optimal choice for immediate-release drug delivery.

Table 40: Optimization of LV IR layer

Batch	Batch No.	% releaseat 30 min	DT for IR Layer (sec)
1	BLV1	52.85±0.625	98
2	BLV2	67.23±0.756	75
3	BLV3	73.50±0.985	72
4	BLV4	65.13±0.318	78
5	BLV5	67.89±0.348	72
6	BLV6	72.56±0.589	56
7	BLV7	74.62±0.792	62
8	BLV8	84.28±0.486	36
9	BLV9	95.23±0.943	24

7.8.6 Evaluation of Bilayer tablet

The formulated bilayer tablet, combining the optimized BOA1 SR tablet and BLV9 IR tablet, demonstrated a hardness of 5.9 kg/cm², indicating sufficient mechanical strength to withstand handling and storage.

The friability was recorded at 0.24%, reflecting excellent resistance to mechanical stress. This minimal friability ensures the tablet remains intact throughout processing and transportation. Overall, the bilayer tablet exhibits both durability and stability, making it appropriate for practical use.

7.8.7 Dissolution study of bilayer Tablet

The dissolution study confirms a controlled and sustained release of oleanolic acid from the SR layer, resulting in a high cumulative drug release by the study's conclusion (Fig. 31). By 12 hours, the formulation successfully delivers 98.34% of the drug, demonstrating its ability to maintain a prolonged release (Table 41). This release pattern highlights the effectiveness of the optimized bilayer formulation in ensuring extended drug delivery.

For the BLV9 formulation, the study shows a rapid and nearly complete release of Lovastatin. Within 30 minutes, approximately 101.09% of the drug is released (Table 42), emphasizing its suitability for immediate release applications. This quick dissolution (Fig. 32) enhances bioavailability and facilitates a faster therapeutic effect, making the optimized formulation ideal for immediate drug delivery.

The bilayer tablet formulation successfully achieved a biphasic drug release pattern, with immediate release of lovastatin followed by sustained release of oleanolic acid. The compression integrity of the bilayer tablet prevented layer interference, ensuring independent functionality of each layer. The rapid release from the IR layer did not influence the sustained release behavior of the SR layer, confirming effective spatial separation and formulation compatibility

Table 41: Dissolution study of OA from bilayer tablet

Time (h)	Cum % drug release of Oleanolic acid				Std Dev.
	I	II	II	Avg	
1	15.31	16.49	17.46	16.42	0.87
2	21.17	20.24	19.28	20.23	0.77
3	29.29	28.18	27.94	28.47	0.58
4	39.21	41.33	40.24	40.26	0.86
6	64.91	63.57	65.89	64.79	0.95
8	85.4	87.94	87.12	86.82	1.05
10	95.14	94.43	93.57	94.38	0.64
12	99.11	98.41	97.50	98.34	0.65

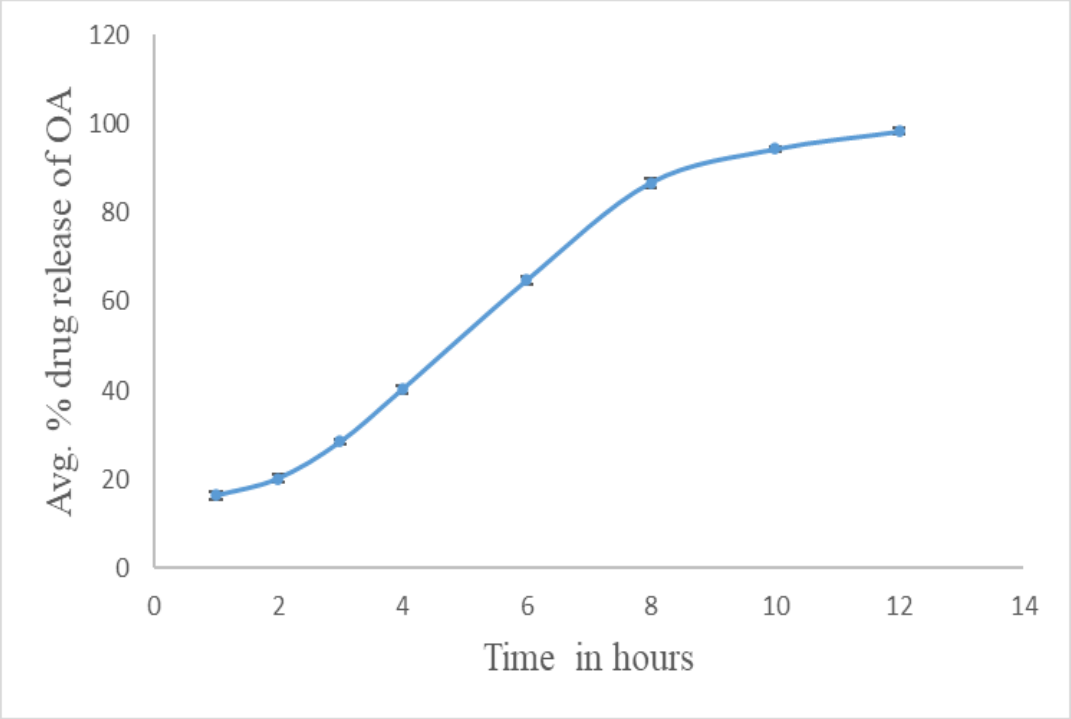

Fig. 31: Drug release of OA from bilayer tablet

Table 42: Dissolution study of LV from bilayer tablet

Time (min)	Cum % drug release of Lovastatin				Std Dev.
	I	II	II	Avg	
10	32.13	34.51	33.8	33.48	0.99
20	63.66	62.4	61.2	62.42	1.00
30	92.95	93.54	94.34	93.61	0.56
60	96.12	97.24	97.49	96.95	0.59
90	97.4	99.09	98.29	98.26	0.69
120	98.37	100.71	100.38	99.82	1.03
150	101.14	100.36	99.16	100.22	0.81
180	99.97	101.11	102.19	101.09	0.90

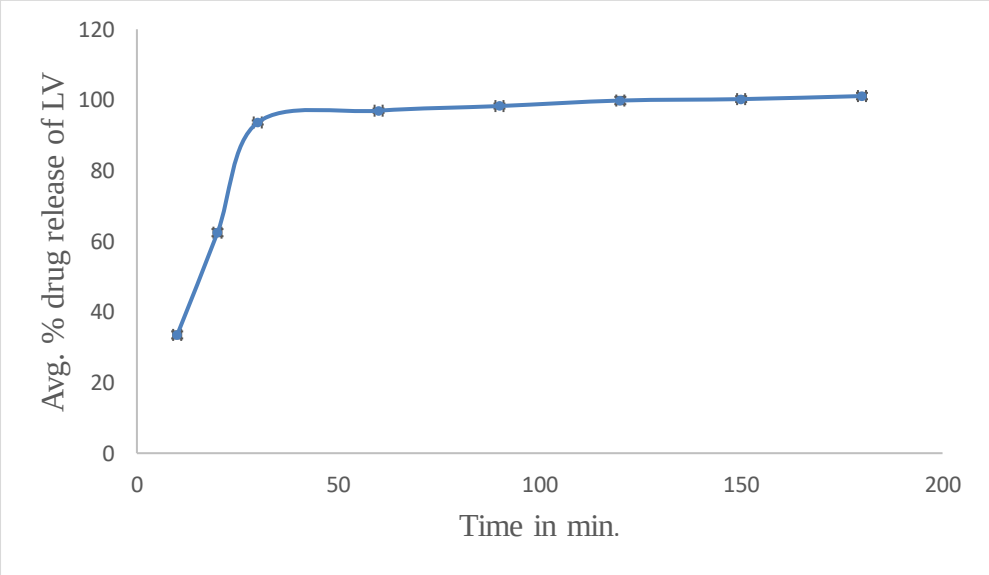

Fig. 32: Drug release of LV from bilayer tablet

7.9 Drug Release Kinetics

7.9.1 Drug Release Kinetics of OA1 batch of core and coat tablet:

The drug release profile of optimized OA1 batch of core and coat tablet presented in the table 43, suggests a controlled and sustained release over 12 hours, with an initial slower release followed by a more pronounced increase in drug dissolution. The regression coefficients (R^2) provide insight into how well the drug release data fit each kinetic model.

Table 43: Drug Release Kinetics Data for Optimized Batch (OA1) of Core and Coat Tablet

Time (h)	% Cumulative Drug Release (CDR)	% Cumulative Drug Release Remaining (CDR RE)	Log (% CDR)	Log (%CDR RE)	Square Root (t)	Log (t)	% Drug Released (DR)	Log (% DR)
0	0	100	0	2	0	0	0	0
1	15.18	84.82	1.18	1.92	1.00	0.00	15.18	1.18
2	19.84	80.16	1.29	1.90	1.41	0.30	19.84	1.29
3	28.45	71.55	1.45	1.85	1.73	0.47	28.45	1.45
4	39.74	60.26	1.59	1.78	2.00	0.60	39.74	1.59
6	65.41	34.59	1.81	1.53	2.44	0.77	65.41	1.81
8	84.32	15.68	1.92	1.19	2.82	0.90	84.32	1.92
10	92.82	7.18	1.96	0.85	3.16	1.00	92.82	1.96
12	96.34	3.66	1.98	0.56	3.46	1.07	96.34	1.98

7.9.1.1 Zero Order Model (R^2=0.982)

This model has the highest R^2 value, indicating a strong linear relationship between cumulative % drug release and time (Fig. 33). This suggests that the drug release from the tablet follows zero-order kinetics, where the release rate is constant over time and is independent of the concentration of the drug.

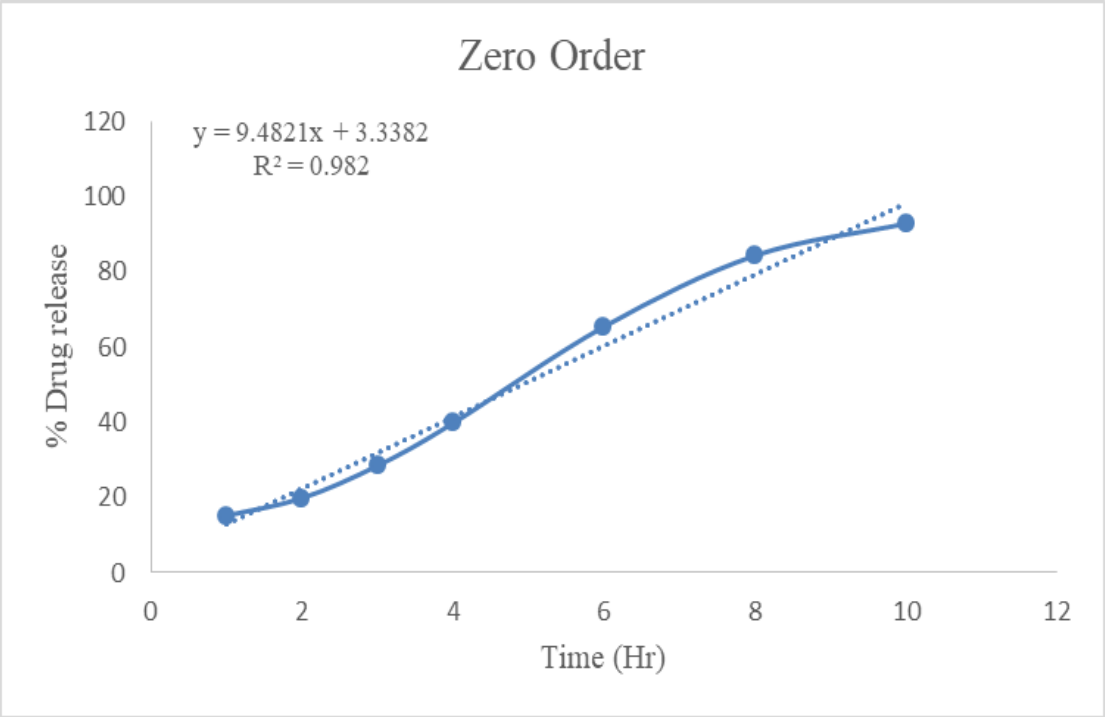

Fig. 33: Zero order drug release kinetics of OA1 batch

7.9.1.2 First Order Model (R2=0.9361)

This model shows a good fit but is not as strong as the zero-order model. The R^2 value indicates that while the release may follow first-order kinetics (Fig. 34), where the release rate depends on the concentration of the drug, it is fewer representatives compared to zero-order.

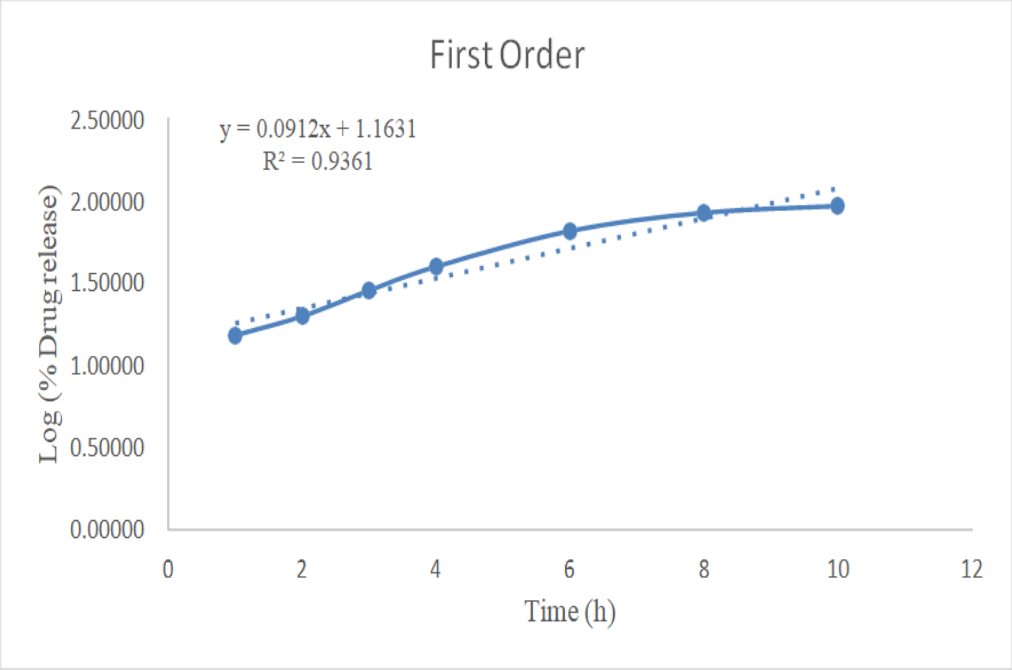

Fig. 34: First order drug release kinetics of OA1 batch

7.9.1.3 Higuchi Model (R^2=0.9652)

The R^2 value for the Higuchi model (Fig. 35) suggests that the drug release could also be influenced by diffusion from a matrix. However, the fit is not as good as the zero-order model.

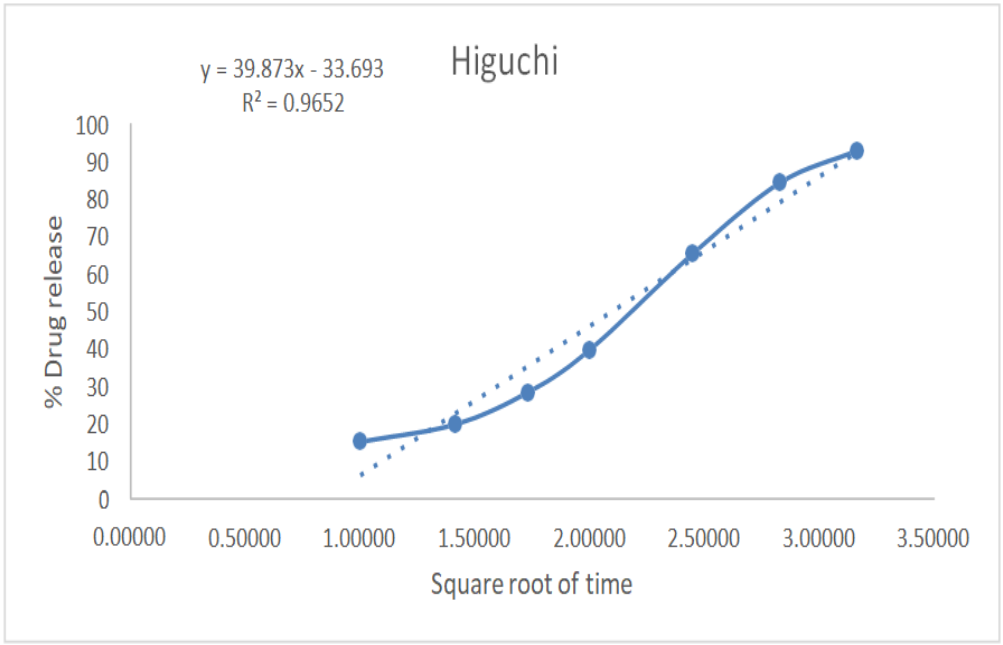

Fig. 35: Higuchi model of OA1 batch

7.9.1.4 Korsmeyer–Peppas Model (R^2=0.9685)

This model indicates that the drug release may follow a complex release mechanism. The R^2 value shows (Fig. 36) a reasonably good fit, but still, the zero-order model provides a better fit for the data.

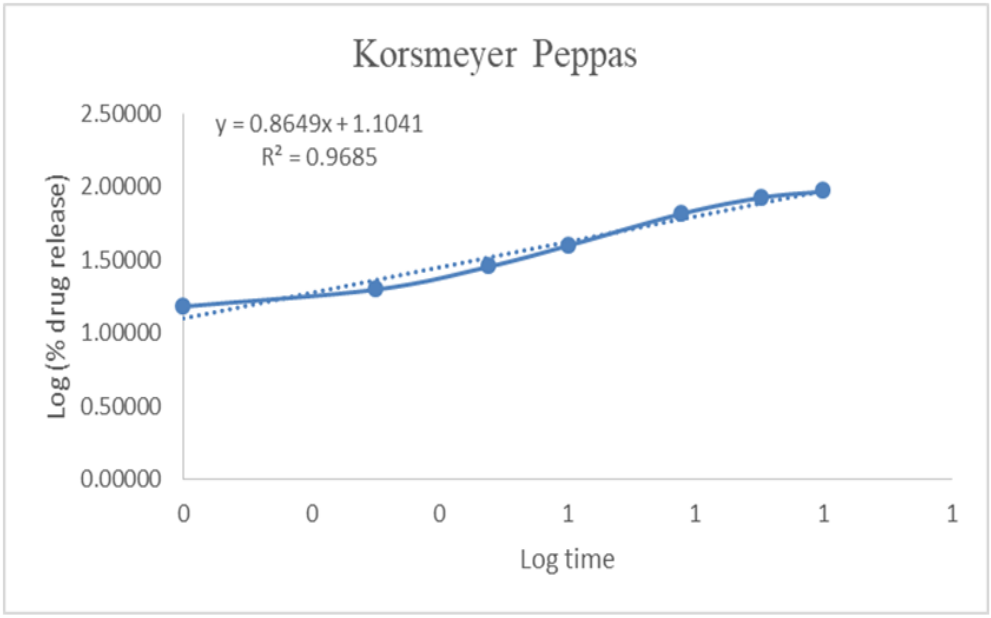

Fig. 36: Korsmeyer–Peppas model of OA1 batch

The highest R^2 value for the zero-order model suggests that the optimized core and coat tablet (OA1) primarily follows zero-order kinetics. This implies that the release rate of the drug is consistent over time, making this model the most probable representation of the drug release behavior for this formulation.

7.9.2 Drug Release Kinetics of LV9 batch of core and coat tablet

The drug release data for LV9 (Table 44), suggests a rapid release of the drug in the initial phase followed by a slower, more controlled release as time progresses. This pattern may indicate a complex release mechanism, possibly involving both diffusion and dissolution processes. The regression coefficient (R^2) values indicate how well the release data of the LV9 formulation fits each kinetic model.

Drug release kinetic analysis revealed that the optimized OA1 formulation followed zero-order kinetics, indicating a constant drug release rate independent of concentration. This behavior is desirable for sustained-release formulations as it ensures prolonged therapeutic levels. In contrast, the LV9 formulation showed rapid initial release, consistent with immediate-release design objectives. These findings confirm

that formulation strategy and excipient selection directly influenced the release kinetics of both layers.

Table 44: Drug Release Kinetics of Optimized Core and Coat Tablet (LV9)

Time (h)	% CDR	% CDR RE	Log (% CDR)	Log (% CDR RE)	Sq Rt (t)	Log (t)	% DR	Log (% DR)
0.17	31.76	68.24	1.50	1.83	0.41	-0.77	31.76	1.50
0.33	61.23	38.77	1.79	1.59	0.57	-0.48	61.23	1.79
0.5	91.24	8.76	1.96	0.94	0.71	-0.30	91.24	1.96
1	97.16	2.84	1.99	0.45	1.00	0.00	97.16	1.99

7.9.2.1 Zero Order (R^2 = 0.7249)

This model suggests that the drug release is constant over time. While the R^2 value (Fig. 37) indicates a reasonable fit, it is not the best fit among the models considered.

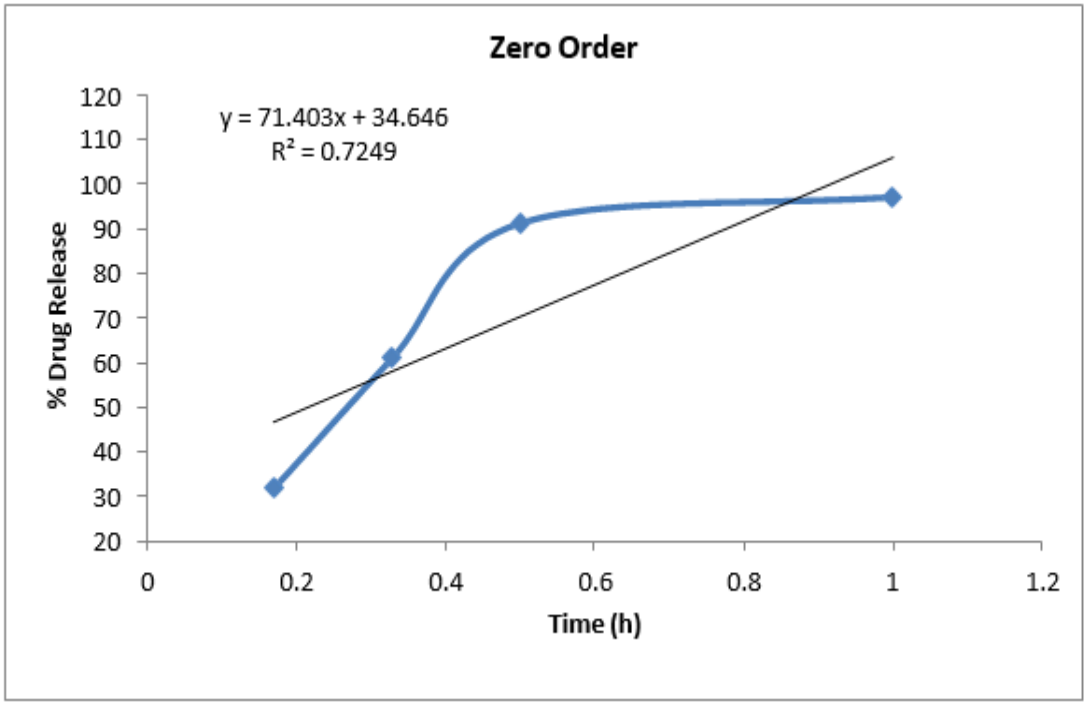

Fig. 37: Zero order drug release kinetics of LV9 batch

7.9.2.2 First Order (R^2 = 0.6513)

This model indicates that the drug release rate is proportional to the remaining drug concentration (Fig. 38). The lower R^2 value suggests that this model is less appropriate for describing the drug release kinetics of LV9 compared to the other models.

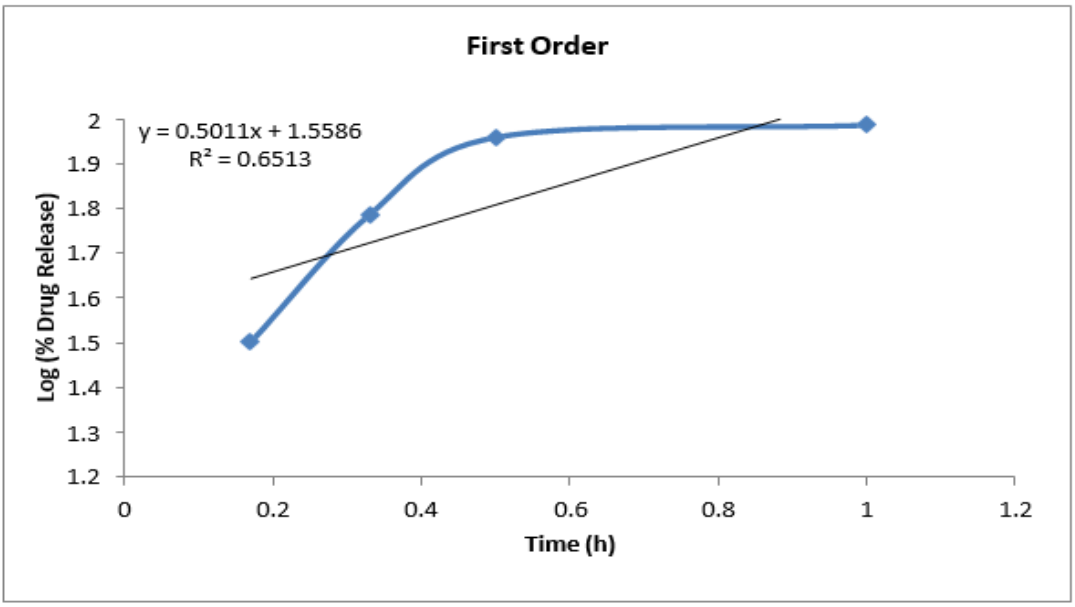

Fig. 38: First order drug release kinetics of LV9 batch

7.9.2.3 Higuchi Model (R^2 = 0.8244)

This model is used for drug release from a matrix system, where the release is influenced by diffusion through a swelling matrix. The R^2 value (Fig. 39) indicates a good fit, suggesting that the Higuchi model describes the drug release kinetics well.

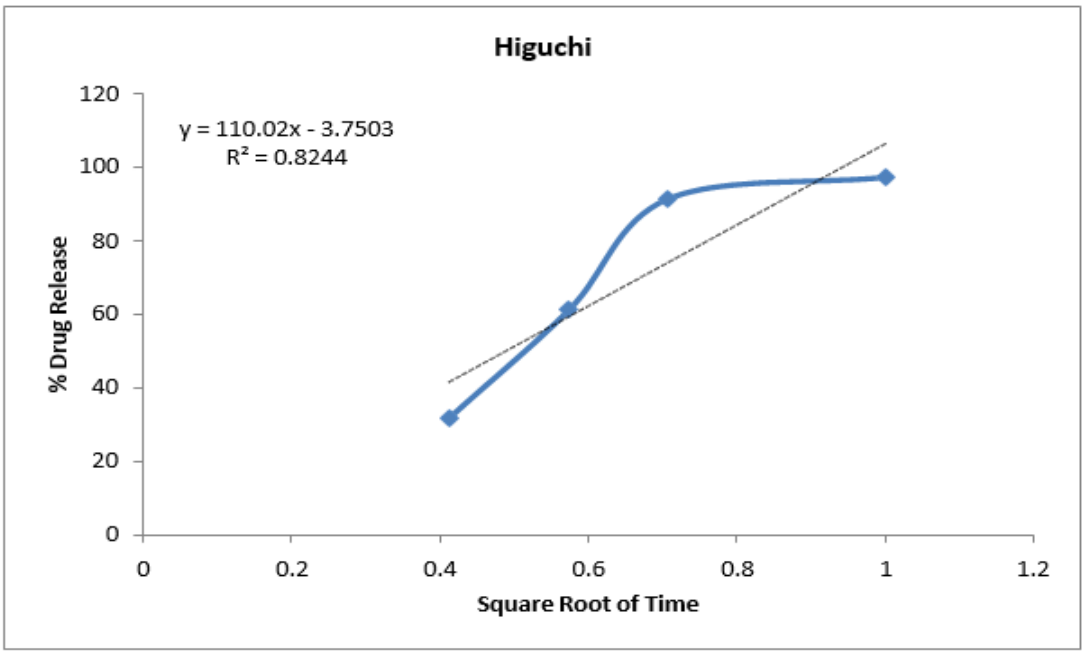

Fig. 39: Higuchi model of LV9 batch

7.9.2.4 Korsmeyer–Peppas Model (R^2 = 0.8704)

This model describes drug release from a polymeric system and accounts for complex release mechanisms involving both diffusion and erosion (Fig. 40). The highest R^2 value among the models suggests that this model best fits the data, indicating that the drug release from the LV9 formulation is most accurately described by the Korsmeyer–Peppas model.

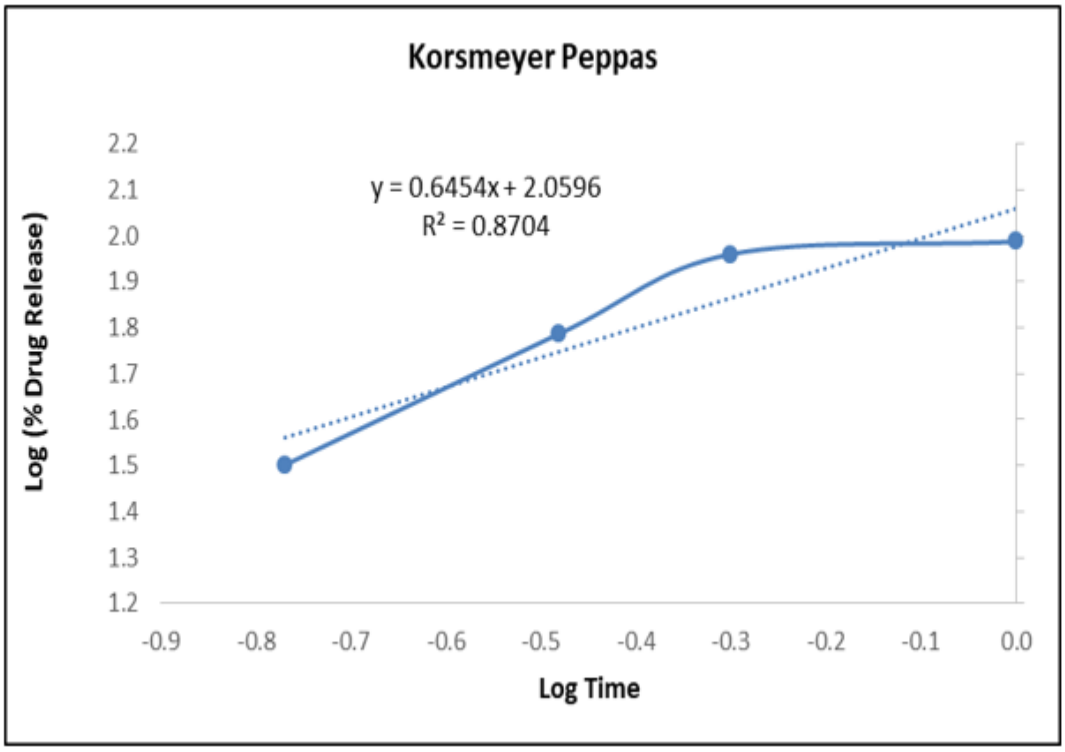

Fig. 40: Korsmeyer–Peppas model of LV9 batch

For the LV9 formulation, the Korsmeyer–Peppas model (R^2 = 0.8704) provides the best fit for the *in vitro* release data, suggesting that the drug release mechanism is best explained by this model. This indicates a more complex release profile involving multiple mechanisms, likely including both diffusion and erosion.

7.9.3 Drug Release Kinetics of SR layer (BOA1) of bilayer tablet

The data shown in Table 45, suggest that BOA1 follows a controlled release mechanism with the drug being steadily released over time. Analysis of the kinetic models reveals which model best describes the release profile, aiding in the optimization of the bilayer tablet formulation.

Table 45: Drug Release Kinetics Data for Optimized Batch (BOA1)

Time (h)	% CDR	% CDR RE	Log (% CDR)	Log (% CDR RE)	Sq Rt (t)	Log (t)	% DR	Log (% DR)
1	16.42	83.58	1.21	1.92	1.00	0.00	16.42	1.21
2	20.23	79.77	1.30	1.90	1.41	0.30	20.23	1.30
3	28.47	71.53	1.45	1.85	1.73	0.47	28.47	1.45
4	40.26	59.74	1.60	1.77	2.00	0.60	40.26	1.60
6	64.79	35.21	1.81	1.54	2.44	0.77	64.79	1.81
8	86.82	13.18	1.93	1.12	2.82	0.90	86.82	1.93
10	94.38	5.62	1.97	0.75	3.16	1.00	94.38	1.97
12	98.34	1.66	1.99	0.22	3.16	1.07	98.34	1.99

7.9.3.1 Zero-Order Model (R^2 =0.9809)

The high R^2 value indicates that the zero-order model fits the release data well (Fig. 41). This model suggests that the drug release from the tablet is constant over time, with the release rate being independent of the amount of drug remaining in the tablet.

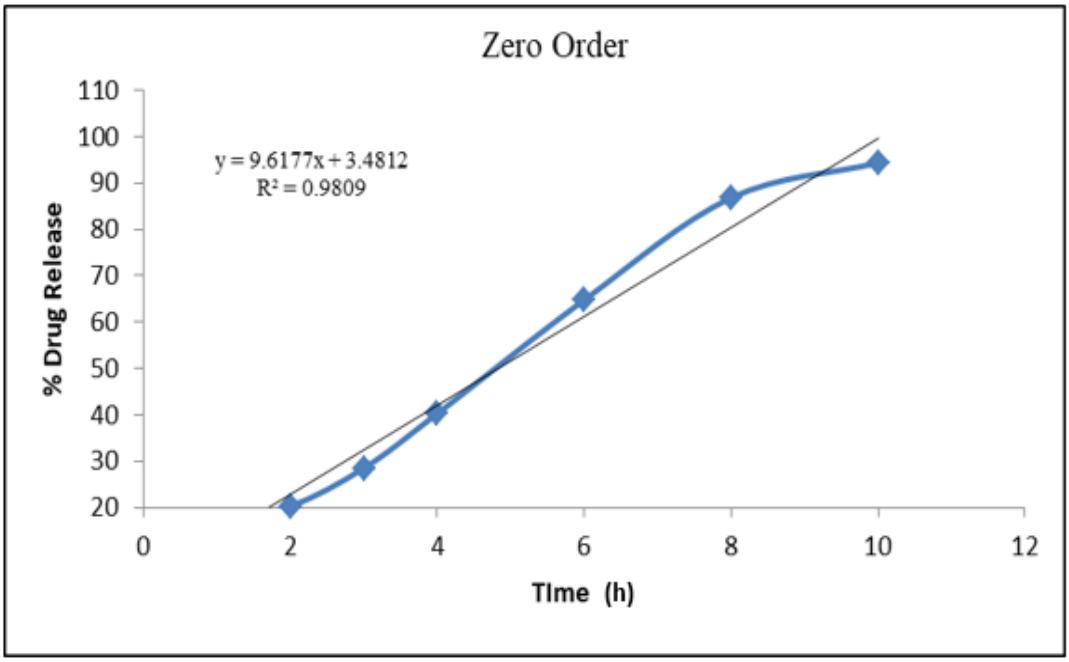

Fig. 41: Zero order drug release kinetics of BOA1 batch

7.9.3.2 First-Order Model (R^2=0.9440)

Although the first-order model also fits the data, its R^2 value is lower than that of the zero-order model (Fig. 42). This model implies that the drug release rate decreases over time as the amount of drug in the tablet decreases.

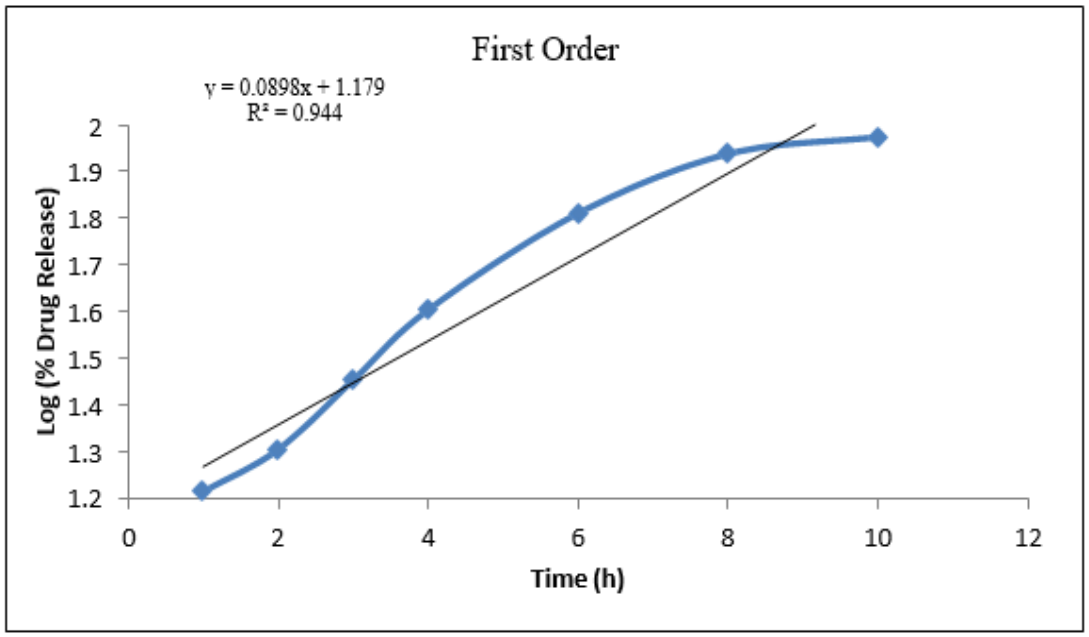

Fig. 42: Zero order drug release kinetics of BOA1 batch

7.9.3.3 Higuchi Model (R^2=0.9589)

The Higuchi model fits the data relatively well (Fig. 43), indicating that the drug release could be controlled by diffusion through the matrix. The R^2 value reflects a good fit for release controlled by diffusion.

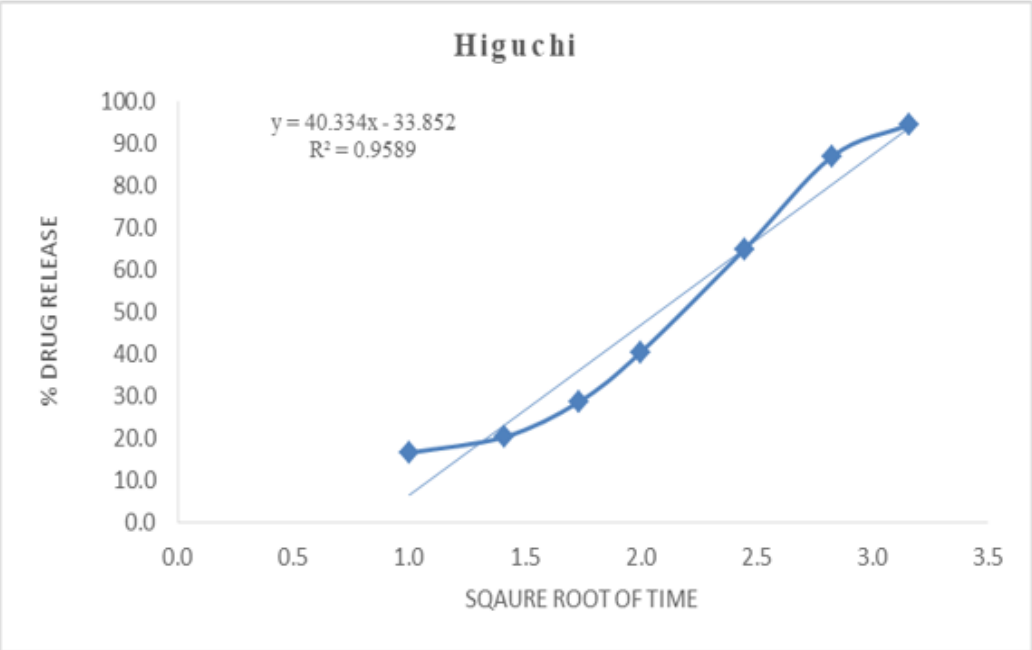

Fig. 43: Higuchi model of BOA1 batch

7.9.3.4 Korsmeyer–Peppas Model (R^2=0.9580)

The Korsmeyer–Peppas model also provides a good fit, suggesting that the release mechanism may involve both diffusion and polymer relaxation (Fig. 44). The R^2 value is like that of the Higuchi model, indicating a potential mixed release mechanism.

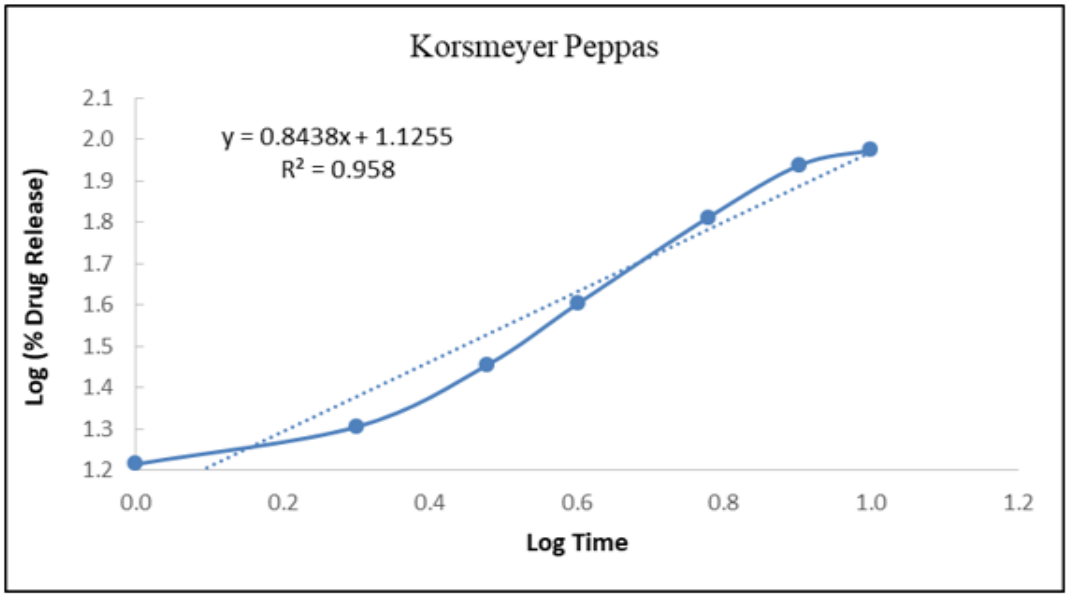

Fig. 44: Korsmeyer–Peppas model of BOA1 batch

For the optimized bilayer tablet batch BOA1, the zero-order release model with an R^2 value of 0.9809 is the most probable model. This suggests that the drug release from this formulation occurs at a constant rate, independent of the concentration of the drug remaining in the tablet. The zero-order kinetics provide a controlled and predictable release profile, which is desirable for maintaining consistent drug levels over time.

7.9.4 Drug Release Kinetics of IR layer (BLV9) of bilayer tablet

The data indicates a potentially rapid dissolution rate (Table 46), which is consistent with the high levels of sodium starch glycolate and croscarmellose sodium used in the formulation. These excipients are known for their ability to facilitate quick disintegration and dissolution.

Table 46: Drug Release Kinetics Data IR layer BLV9

Time (Hr)	% CDR	% CDR RE	Log (% CDR)	Log (% CDR RE)	Sq Rt (t)	Log (t)	% DR	Log (% DR)
0.17	33.48	66.520	1.525	1.823	0.412	-0.770	33.480	1.525
0.33	62.42	37.580	1.795	1.575	0.574	-0.481	62.420	1.795
0.5	93.61	6.390	1.971	0.806	0.707	-0.301	93.610	1.971
1	96.95	3.050	1.987	0.484	1.000	0.000	96.950	1.987

7.9.4.1 Zero-Order Model (R^2 =0.6959)

The Zero-Order model (Fig. 45) suggests that the drug release occurs at a constant rate, independent of concentration. However, the R^2 value (0.6959) indicates a moderate correlation, meaning that while the model somewhat fits the data, there are deviations, implying that the release mechanism may not strictly follow zero-order kinetics. The drug release is not entirely governed by a zero-order process, suggesting other mechanisms may be involved.

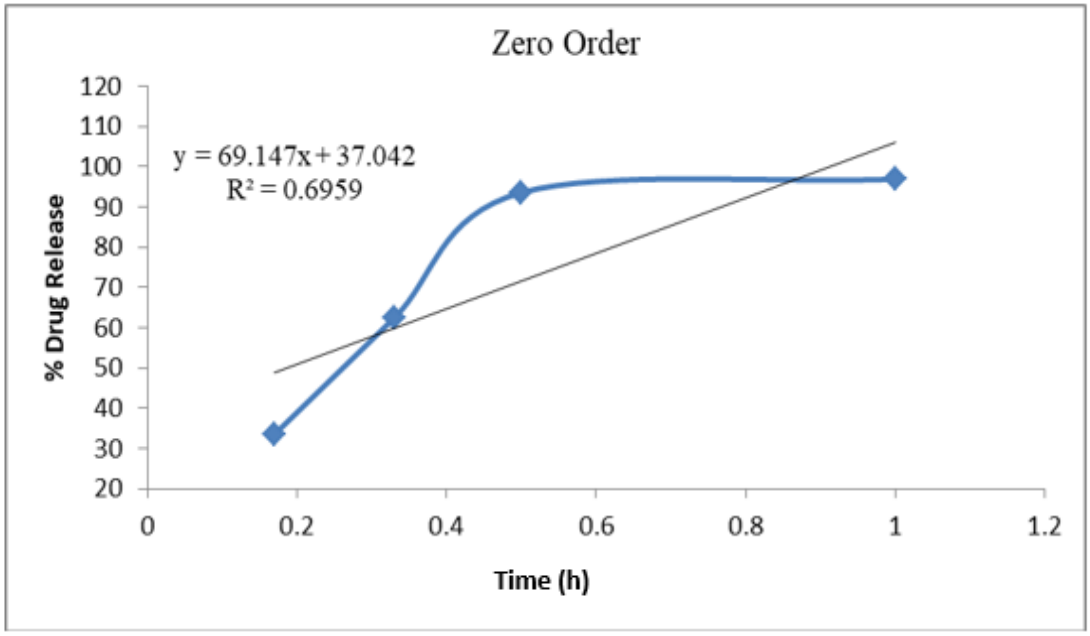

Fig. 45: Zero order drug release kinetics of BLV9 batch

7.9.4.2 First-Order Model (R^2 = 0.6369)

This model assumes that the release rate is dependent on the remaining drug concentration (Fig. 46). The R^2 value (0.6369) indicates a weaker fit compared to the Zero-Order model, meaning the release does not strongly follow first-order kinetics. The drug release is not primarily controlled by first-order kinetics, as the correlation is relatively low.

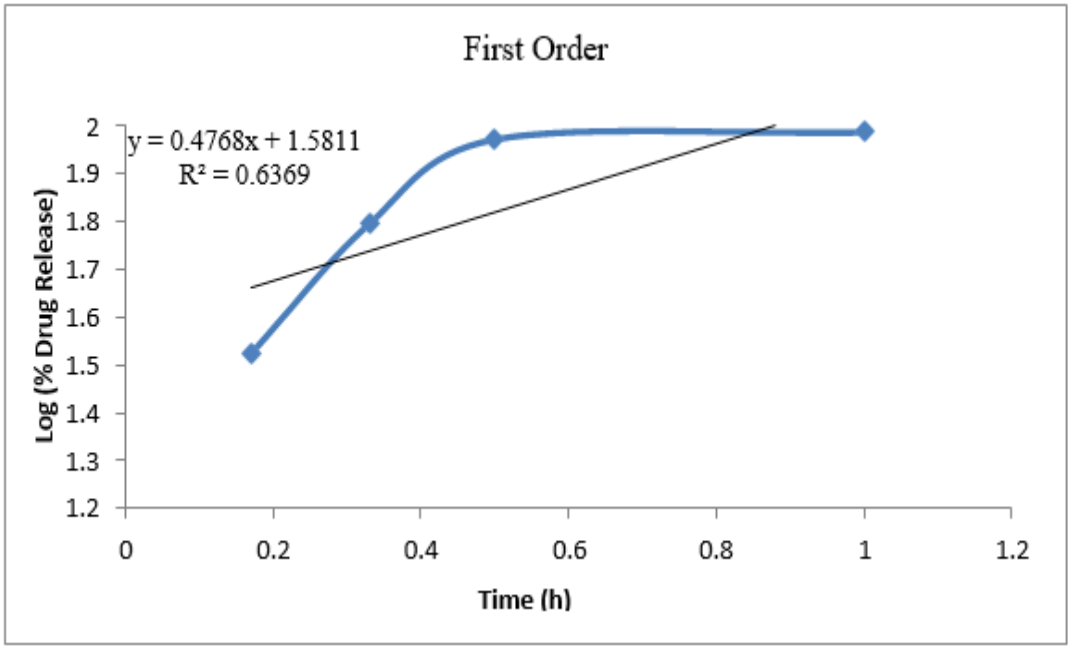

Fig. 46: First order drug release kinetics of BLV9 batch

7.9.4.3 Higuchi Model (R^2 = 0.7987)

The Higuchi model assumes drug release occurs through diffusion from a matrix system. The relatively high R^2 value (0.7987) suggests that diffusion plays a significant role in drug release but may not be the sole mechanism (Fig. 47). The drug release is well-explained by the Higuchi model, indicating that diffusion is a key factor, but other processes might also be involved.

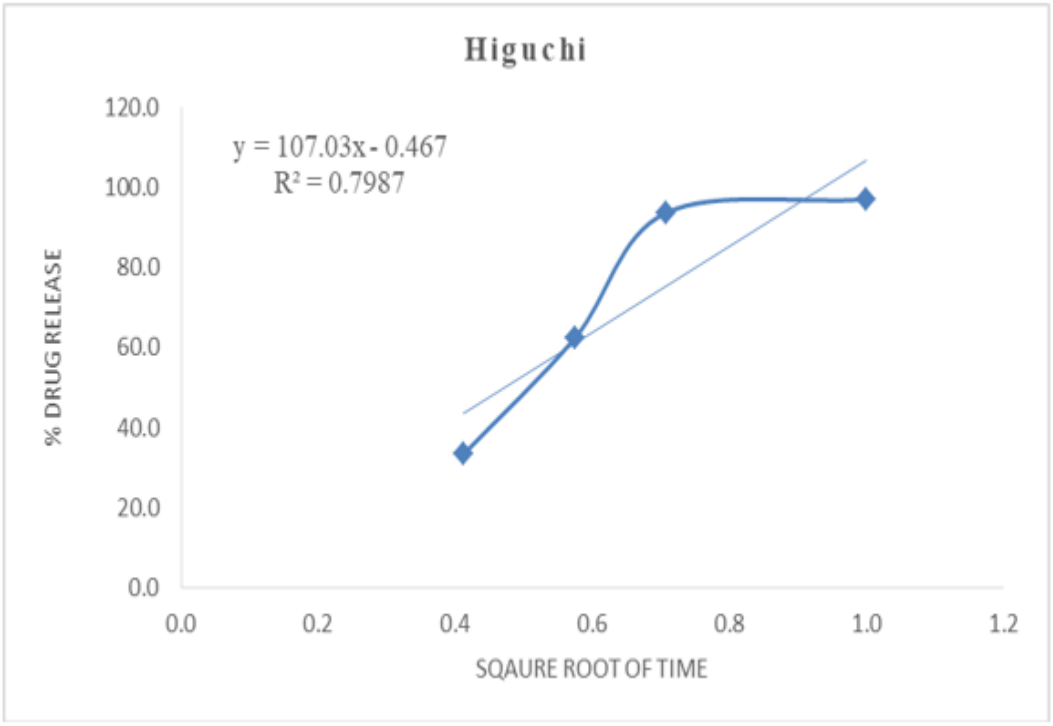

Fig. 47: Higuchi model of BLV9 batch

7.9.4.4 Korsmeyer–Peppas Model (R^2 = 0.8590)

The Korsmeyer–Peppas model is often used to describe drug release from polymeric systems, indicating whether the mechanism follows diffusion, swelling, or erosion. The high R^2 value (0.8590) suggests a strong correlation, meaning the model effectively explains the release behaviour (Fig. 48). This model provides the best fit among the four, suggesting that the release mechanism is likely governed by a combination of diffusion and polymer relaxation or swelling.

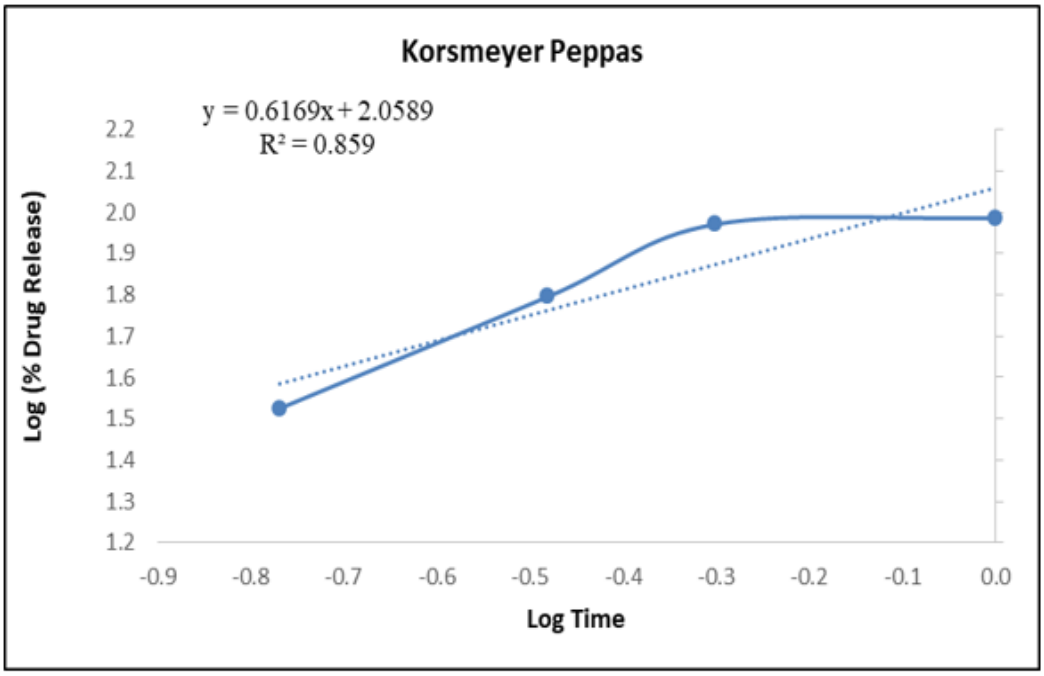

Fig. 48: Korsmeyer–Peppas model of BOA1 batch

For the optimized BLV9 batch of bilayer, the Korsmeyer–Peppas model (R^2 = 0.8590) provides the best fit, suggesting that the drug release mechanism is complex and may involve both diffusion and polymeric interactions rather than following simple zero-order or first-order kinetics. The Higuchi model (R^2 = 0.7987) also shows a strong correlation, indicating that diffusion significantly contributes to the release process. (Table 47)

Table 47: Kinetic Modeling of Drug Release from Optimized Formulations

Formulation	**Zero-order (R^2)**	**First-order (R^2)**	**Higuchi (R^2)**	**Korsmeyer–Peppas (R^2)**	***n* value**	**Best-fit Model**
BLV9 (IR layer – Lovastatin)	0.842	0.914	0.931	0.967	0.610	Korsmeyer–Peppas
Optimized SR layer (Oleanolic acid)	0.962	0.887	0.984	0.978	0.720	Higuchi

Comparison with Reported and Marketed Formulations

Previously reported sustained-release formulations of oleanolic acid have demonstrated incomplete drug release or non-linear release profiles beyond 8–10 hours due to poor

aqueous solubility and matrix erosion limitations. In comparison, the optimized formulation in the present study achieved ~98% drug release over 12 hours with zero-order kinetics, indicating improved release control and formulation efficiency.

Marketed lovastatin immediate-release tablets typically achieve rapid drug release within 30–45 minutes; however, variability in disintegration time has been reported. The optimized IR layer (BLV9) in the present study achieved >95% drug release within 30 minutes, comparable to marketed formulations, while being successfully integrated into a bilayer system.

Unlike conventional monolayer or single-drug formulations reported in literature, the present bilayer tablet provides a dual-release system combining immediate lipid-lowering action with sustained antioxidant and antihyperlipidemic support, highlighting its therapeutic advantage and formulation novelty.

7.10 Stability Studies

7.10.1 Stability Study of Optimized Batch of Core and Coat Tablets:

7.10.1.1 Physical Appearance

The tablets maintained a consistent white color throughout the testing period, indicating no significant physical changes.

7.10.1.2 Weight Variation

The weight variation of the tablets remained stable across all testing intervals, showing minor fluctuations within acceptable limits.

7.10.1.3 Hardness

Hardness values remained relatively stable, with minor variations over the6-month period. This suggests that the tablets' mechanical strength is well- preserved under accelerated conditions.

7.10.1.4 Drug Content

Drug content for both Oleanolic Acid (OA) and Lovastatin (LV) remained consistent, with slight variations. The percentage of drug content is well within acceptable limits, indicating stability of the active ingredients.

7.10.1.5 Friability

Friability increased slightly over the 6-month period but remains within acceptable limits, indicating that the tablets' resistance to abrasion has not been significantly affected.

Table 48: Stability study of optimized batch of core and coat tablets

Time	Physical appearance	Weight Variation	Hardness (kg/cm^2)	Drug Content (%)		Friability (%)
				OA	LV	
Initial	White	305.400 ± 1.586	5.440 ± 0.049	99.511	100.208	0.091
1st Month	White	305.500 ± 1.558	5.380 ± 0.075	99.631	100.059	0.091
3rd Month	White	305.450 ± 1.527	5.500 ± 0.110	99.394	99.800	0.115
6th Month	White	305.300 ± 1.384	5.360 ± 0.080	99.454	99.963	0.124

The accelerated stability study summarized in Table 48, demonstrates that the core and coat tablets maintain their quality, with minimal changes in physical appearance, weight variation, hardness, drug content, and friability over a 6-month period at accelerated conditions. This suggests that the tablets are likely to be stable under normal storage conditions for the recommended shelf life.

7.10.2 Stability Study of Optimized Batch of Bilayer Tablets

7.10.2.1 Physical Appearance

The tablets retained their white color throughout the study, indicating no significant physical changes.

7.10.2.2 Weight Variation

The weight variation of the tablets remained stable across all time points, showing minimal fluctuation within acceptable limits.

7.10.2.3 Hardness

Hardness values showed minor variations but remained within the acceptable range, indicating good mechanical strength of the tablets over the study period.

7.10.2.4 Drug Content

The drug content for both Oleanolic Acid (OA) and Lovastatin (LV) remained consistent, with slight variations. The content levels are within acceptable limits, suggesting stability of the active ingredients.

7.10.2.5 Friability

Friability increased slightly over the 6-month period but remains within acceptable limits. This indicates that the tablets' resistance to abrasion has not significantly deteriorated.

Table 49: Stability study of optimized batch of bilayer tablets

Time	Physical appearance	Weight Variation	Hardness (kg/cm^2)	Drug Content(%)		Friability (%)
				OA	LV	
Initial	White	305.350 ± 1.476	5.460 ± 0.058	100.127	100.171	0.103
1st Month	White	305.400 ± 1.346	5.420 ± 0.055	100.044	100.101	0.114
3rd Month	White	305.500 ± 1.625	5.500 ± 0.116	99.545	99.992	0.121
6th Month	White	305.253 ± 1.297	5.360 ± 0.124	99.377	99.706	0.121

The stability study of the optimized bilayer tablets (Table 49) shows that they maintain their quality and performance over 6 months under accelerated conditions. There are minimal changes in physical appearance, weight variation, hardness, drug content, and friability, suggesting the tablets are stable and appropriate for the intended shelf life.

Techniques

7.11 Pharmacodynamic study

7.11.1 Effect on Total Cholesterol (TC) level

The normal control group showed a mean total cholesterol level of 58.67 ± 9.33 mg/dl. The disease control group, which was fed a high-fat diet, showed a significantly elevated mean total cholesterol level of 594.7 ± 21.33 mg/dl ($p<0.0001$) compared to the normal control. Rabbits treated with Lovastatin (API) at a dose of 0.52 mg/kg showed a significant reduction in total cholesterol levels (111.67 ± 19.91 mg/dl; $p<0.0001$) compared to the disease control group. Test Compound A and Test Compound B also significantly reduced total cholesterol levels to 238.33 ± 52.10 mg/dl and 182.17 ± 42.7 mg/dl, respectively ($p<0.0001$), when compared to the disease control group. These results indicate that both Lovastatin and the test compounds significantly reduce total cholesterol levels in high-fat diet-induced hyperlipidemia in rabbits (Fig. 49).

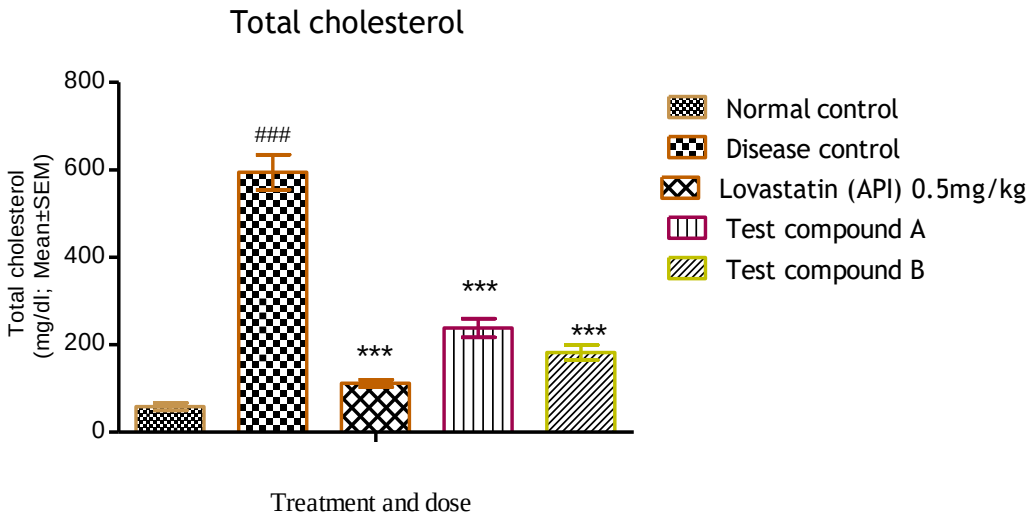

Fig. 49: Effect of treatment on TC level of each group

7.11.2 Effect on TG level

In the study, the normal control group exhibited a mean triglyceride level of 50.5 ± 2.93 mMol/L. The disease control group, subjected to a high-fat diet, showed a markedly elevated mean triglyceride level of 214 ± 18.43 mMol/L ($p<0.0001$) compared to the normal control. Treatment with Lovastatin (API) at a dose of 0.52 mg/kg resulted in a significant reduction in triglyceride levels to 126.17 ± 27.13 mMol/L ($p<0.0001$).

Test Compound A and Test Compound B also led to significant decreases in triglyceride levels, with mean values of 143.33 ± 25.41 mMol/L and 116.63 ± 13.16 mMol/L, respectively ($p<0.0001$) compared to the disease control group. These findings (Fig. 50) demonstrate that both Lovastatin and the test compounds significantly reduce triglyceride levels in high-fat diet-induced hyperlipidemia in rabbits.

7.11.3 Effect on HDL level

In the study, the normal control group had a mean HDL-C level of 34.2 ± 4.3 mMol/L. The disease control group, subjected to a high-fat diet, exhibited a significantly reduced mean HDL-C level of 13.5 ± 2.64 mMol/L ($p<0.001$) compared to the normal control. Treatment with Lovastatin (API) at a dose of 0.52 mg/kg resulted in a HDL-C level of 19.03 ± 6.3 mMol/L, which was not significantly different from the disease control (NS). Test Compound A yielded an HDL-C level of 24.5 ± 5.13 mMol/L, also not significantly different from the disease control (NS). However, Test Compound B

showed a notable improvement with an HDL-C level of 31.5 ± 5.01 mMol/L, which was significantly different from the disease control ($p<0.001$). These results in Fig. 51 indicate that while Test Compound B significantly improved HDL-C levels compared to the disease control, Lovastatin and Test Compounds A did not show significant differences from the disease control group.

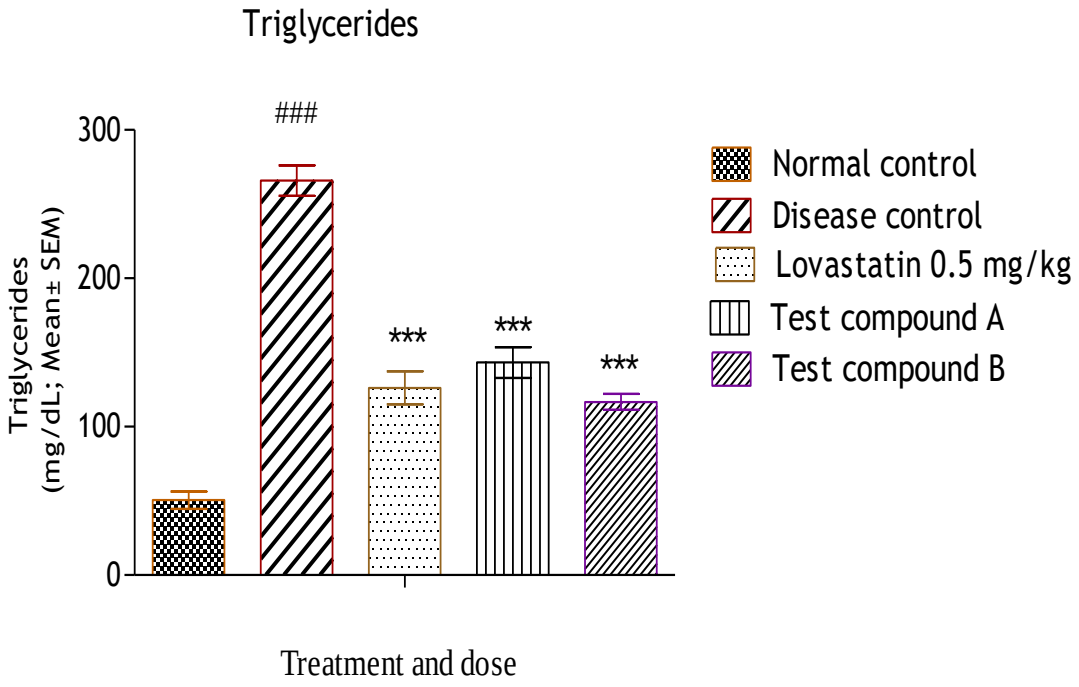

Fig. 50: Effect of treatment on TG level of each group

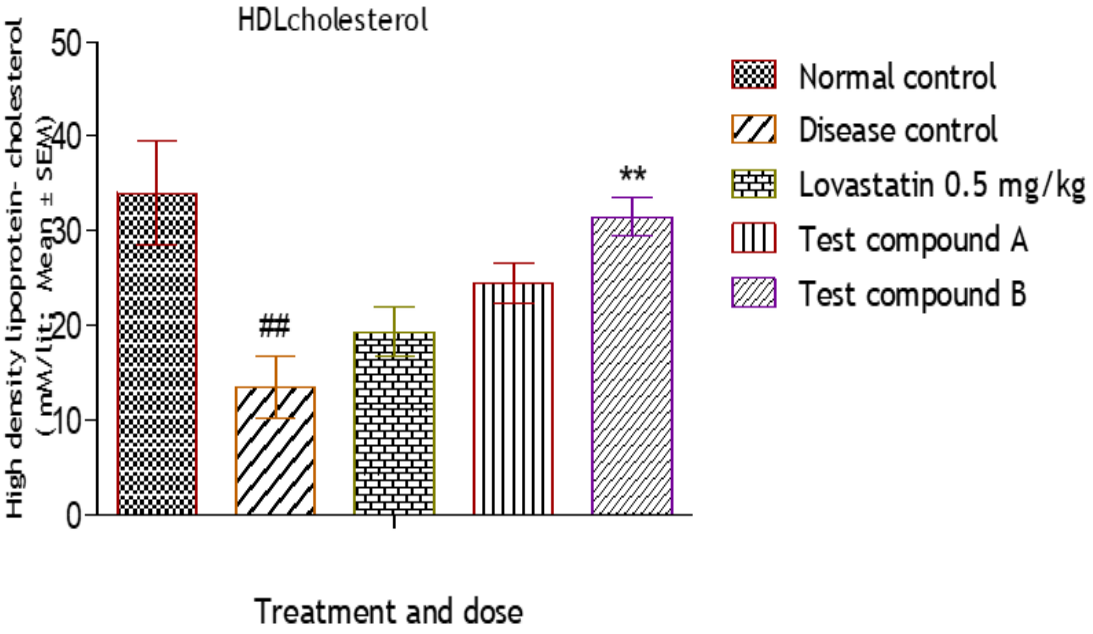

Fig. 51: Effect of treatment on HDL level of each group

Although the in-vivo pharmacodynamic study demonstrated significant reductions in total cholesterol, triglycerides, and LDL-C levels for Lovastatin and the test compounds, some parameters, particularly HDL-C, did not fully reach the anticipated levels in all treatment groups. This may be attributed to formulation-dependent drug release kinetics, individual biological variability among the experimental animals, and the relatively short duration of the study. The sustained release of oleanolic acid from the core layer contributed to prolonged lipid-lowering effects, while the immediate-release layer of Lovastatin ensured early onset of pharmacological action, explaining the observed differential responses across lipid parameters. Despite these partial deviations, the overall results validate the therapeutic potential of the optimized core–coat and bilayer formulations. Future investigations with extended treatment duration, dose optimization, and additional biochemical markers are warranted to comprehensively evaluate the formulations' efficacy and to achieve complete modulation of all lipid parameters, including HDL-C

7.11.4 Effect on LDL-C level

In the study, the normal control group exhibited a mean LDL-C level of 50.17 ± 4.0 mMol/L. The disease control group, which received a high-fat diet, demonstrated a significantly elevated LDL-C level of 608.2 ± 57.54 mMol/L ($p<0.0001$) compared to the normal control. Treatment with Lovastatin (API) at a dose of 0.52 mg/kg resulted in

a mean LDL-C level of 207.8 ± 44.19 mMol/L, significantly reduced compared to the disease control group (p<0.0001). Test Compound A produced an LDL-C level of 215.8 ± 40.62 mMol/L, also significantly lower than the disease control (p<0.0001). Test Compound B showed the lowest LDL-C level at 125.8 ± 23.64 mMol/L, significantly reduced compared to the disease control (p<0.0001). These results indicate that all treatments significantly reduced LDL-C levels compared to the disease control, with Test Compound B being the most effective in lowering LDL-C levels (Fig.52).

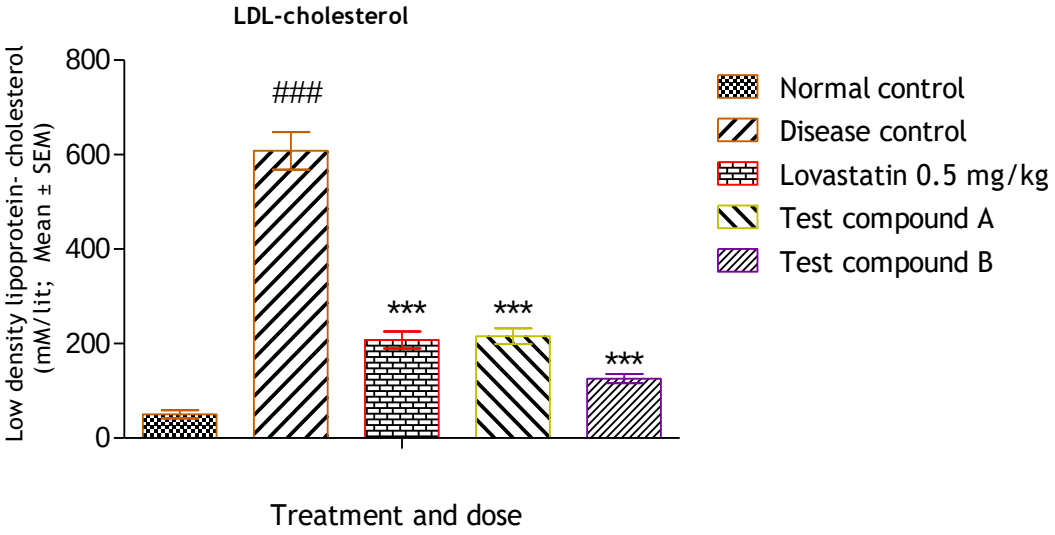

Fig. 52: Effect of treatment on LDL-C level of each group

7.11.5 Effects on Body Weight

Results of the average body weights of each group and change in body weight after treatment was expressed in table 50. The normal control group maintained steady weight gain, while the disease control group, fed a high-fat diet (HFD), exhibited the highest weight gain, confirming the expected impact of an HFD on body weight. The standard treatment group, receiving Lovastatin, showed stable weight progression with minimal fluctuations, suggesting effective lipid regulation without significant weight gain. Test compound A, combining Oleanolic Acid and Lovastatin, led to slight weight reduction or stabilization in later weeks, indicating a potential lipid-lowering effect influencing body weight. Test compound B, using a core-coat tablet formulation, also showing reduction in body weight of rabbits in all groups.

Table 50: Effects on Body Weight on different weeks

Animal Codes	Body weight (kg) at various days			
	First week	Second week	Third week	Fourth week
G1/1	2.50	2.70	2.91	3.20
G1/2	2.70	2.69	2.80	2.93
G1/3	2.60	2.87	3.12	3.31
G1/4	2.58	2.71	2.98	3.21
G1/5	2.70	2.90	3.11	3.39
G1/6	2.90	3.10	3.24	3.52
G2/1	3.00	3.30	3.61	3.90
G2/2	2.60	2.90	3.50	3.80
G2/3	2.73	3.00	3.70	4.00
G2/4	2.67	3.10	3.80	4.10
G2/5	2.71	3.28	3.65	4.07
G2/6	2.90	3.40	3.88	4.10
G3/1	2.83	2.94	3.20	3.40
G3/2	2.75	2.77	2.76	2.78
G3/3	2.66	2.70	2.70	2.70
G3/4	2.89	2.92	2.90	2.89
G3/5	2.55	2.71	2.74	2.73
G3/6	2.91	3.10	3.10	3.10
G4/1	3.10	3.10	2.93	2.94
G4/2	3.00	3.10	3.10	2.94
G4/3	2.95	3.10	3.00	2.99
G4/4	2.86	3.10	2.88	2.78
G4/5	2.64	2.89	2.77	2.88
G4/6	2.70	2.95	2.78	2.69
G5/1	2.73	2.89	2.78	2.85
G5/2	2.78	2.91	3.00	2.83
G5/3	2.80	3.00	2.84.	2.85
G5/4	2.59	2.71	2.72	2.65
G5/5	2.77	2.89	3.27	3.11
G5/6	3.10	3.31	3.32	3.12

The in-vitro dissolution behavior of the optimized formulations closely aligns with the observed in-vivo pharmacodynamic outcomes. The sustained release of oleanolic acid from the SR/core layer ensured prolonged drug availability, which is reflected in the sustained lipid-lowering effect observed in the animal study. Similarly, the rapid in-vitro release of lovastatin from the IR layer resulted in faster drug availability, contributing to the early onset of pharmacodynamic response.

Although a formal Level A IVIVC was not established, the consistent relationship between controlled in-vitro release profiles and enhanced in-vivo efficacy suggests a meaningful in-vitro–in-vivo association. These findings demonstrate that the optimized bilayer/core-coat system successfully translates in-vitro performance into in-vivo therapeutic effectiveness**.**

7.12 Pharmacokinetic study

7.12.1 Pharmacokinetic evaluation by LC-MS/MS system

Detection of pharmacokinetic evaluation was performed via MRM of the transitions: m/z 455.3 to 112.1 for oleanolic acid, m/z 405.4 to 285.3 for lovastatin, and m/z 312.1 to 270.2 for Benorilate (IS). For quantification, peak area ratios of the target ions of the drugs to the internal standard were plotted against their concentrations. MRM ion-chromatograms of LV and OA are shown in Fig. 53 and 54 respectively.

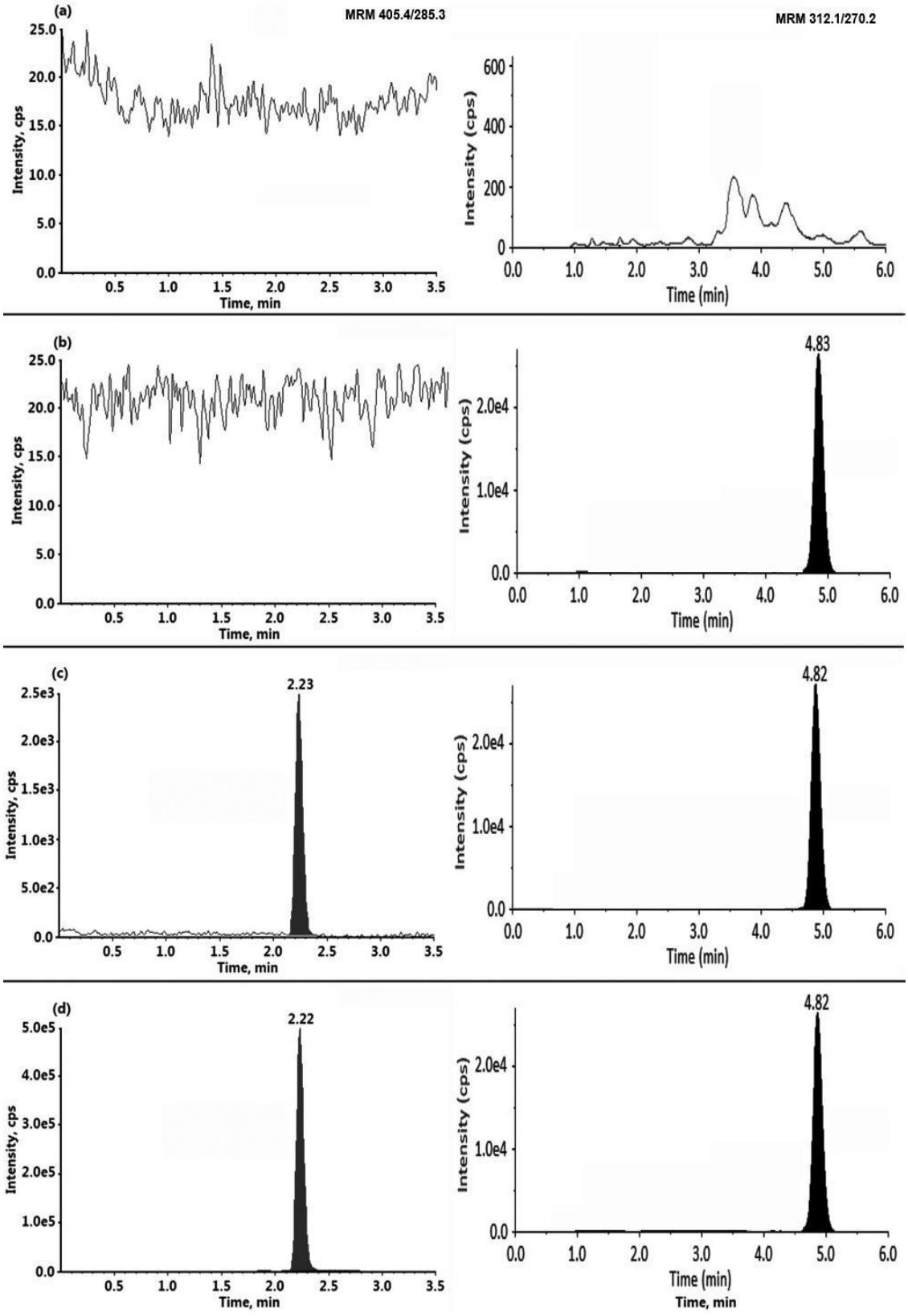

Fig. 53: MRM ion-chromatograms of (a) double blank plasma (without analyte and IS) (b) blank plasma with BN (IS) (c) LV at LLOQ and IS (d) LV at ULOQ and IS

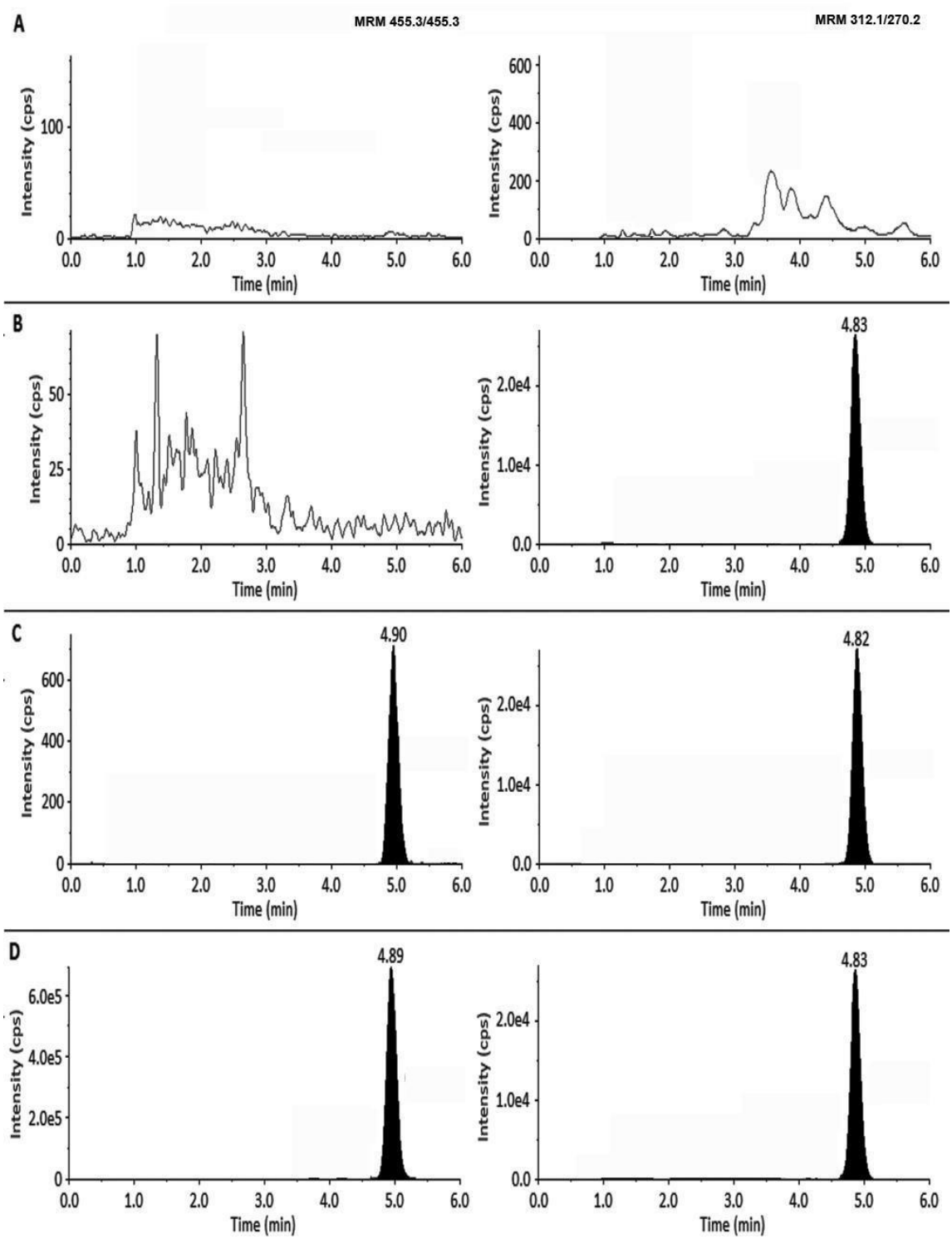

Fig. 54: MRM ion-chromatograms of (a) double blank plasma (without analyte and IS) (b) blank plasma with BN (IS) (c) OA at LLOQ and IS (d) OA at ULOQ and IS

The MRM ion-chromatograms of lovastatin confirm the successful detection and quantification of LV and OA in plasma samples using BN as the internal standard. The absence of peaks in the double blank sample (a) ensures no interference from endogenous compounds. The IS peak in the blank plasma (b) demonstrates its stable retention time and reproducibility. The clear and well-defined peaks at LLOQ (c) and ULOQ (d) indicate the technique's sensitivity and wide dynamic range for quantification of LV and OA. The consistent IS peak across all relevant samples supports the accuracy and reliability of the analytical method.

7.12.2 Linearity of LV by LC-MS/MS system

The linearity of Lovastatin was assessed within the concentration range of 1–10 ng/mL. Each sample was analyzed in triplicate, and the corresponding peak areas were recorded. The response factor was determined by calculating the ratio of the drug's peak area to that of the internal standard (IS). Table 51 and 52 represents the response factor of LV standard and in spiked plasma, respectively. The calibration curve was generated by plotting the response factors against their corresponding concentrations. Fig. 55 and 56 represents the calibration curve for LV standard and in spiked plasma, respectively. The calibration curves for Lovastatin demonstrated strong linearity across the concentration range of 1–10 ng/mL Calibration curve of LV standard and spiked plasma were best fitted by a linear equation, confirming a direct proportional relationship between concentration and response. The coefficient of determination (R^2) values indicating high precision and accuracy in quantification.

Table 51: Response factor for linearity of LV standard

Replica 1				
LV Concentration (ng/mL)	BN Concentration (ng/mL)	LV Area	BN Area	LV Response Factor
1	50	398841	262145	1.521
2	50	486936	264585	1.840
4	50	680791	265879	2.561
6	50	878002	265478	3.307
8	50	1013031	254125	3.986
10	50	1212636	265411	4.569
Replica 2				
LV Concentration (ng/mL)	BN Concentration (ng/mL)	LV Area	BN Area	LV Response Factor
1	50	408812	266077	1.536
2	50	484014	268554	1.802
4	50	676706	265347	2.550
6	50	809952	269460	3.006
8	50	1002153	253617	3.951
10	50	1202952	264880	4.541
Replica 3				
LV Concentration (ng/mL)	BN Concentration (ng/mL)	LV Area	BN Area	LV Response Factor
1	50	396448	261621	1.515
2	50	499109	264056	1.890
4	50	697811	269867	2.586
6	50	870334	264947	3.285
8	50	1058357	257937	4.103
10	50	1290960	269392	4.792
Average Response Factor				
LV Concentration (ng/mL)	Response Factor 1	Response Factor 2	Response Factor 3	Average Response Factor
1	1.521	1.536	1.515	1.524
2	1.84	1.802	1.89	1.844
4	2.561	2.55	2.586	2.566
6	3.307	3.006	3.285	3.199
8	3.986	3.951	4.103	4.013
10	4.569	4.541	4.792	4.634

Table 52: Response factor for linearity of LV in Spiked Plasma

Replica 1				
LV Concentration (ng/mL)	BN Concentration (ng/mL)	LV Area	BN Area	LV Response Factor
1	50	392460	275252	1.426
2	50	496482	278894	1.780
4	50	669898	279173	2.400
6	50	877917	278752	3.149
8	50	1106887	266297	4.157
10	50	1254834	278682	4.503
Replica 2				
LV Concentration (ng/mL)	BN Concentration (ng/mL)	LV Area	BN Area	LV Response Factor
1	50	390890	274701	1.423
2	50	487188	277258	1.757
4	50	667218	278615	2.395
6	50	852124	278194	3.063
8	50	1010783	266564	3.792
10	50	1240615	278125	4.461
Replica 3				
LV Concentration (ng/mL)	BN Concentration (ng/mL)	LV Area	BN Area	LV Response Factor
1	50	398347	274977	1.449
2	50	489145	277814	1.761
4	50	679946	277536	2.450
6	50	857554	278473	3.079
8	50	1084023	266831	4.063
10	50	1188157	278403	4.268
Average Response Factor				
LV Concentration (ng/mL)	Response Factor 1	Response Factor 2	Response Factor 3	Average Response Factor
1	1.426	1.423	1.449	1.433
2	1.780	1.757	1.761	1.766
4	2.400	2.395	2.450	2.415
6	3.149	3.063	3.079	3.097
8	4.157	3.792	4.063	4.004
10	4.503	4.461	4.268	4.411

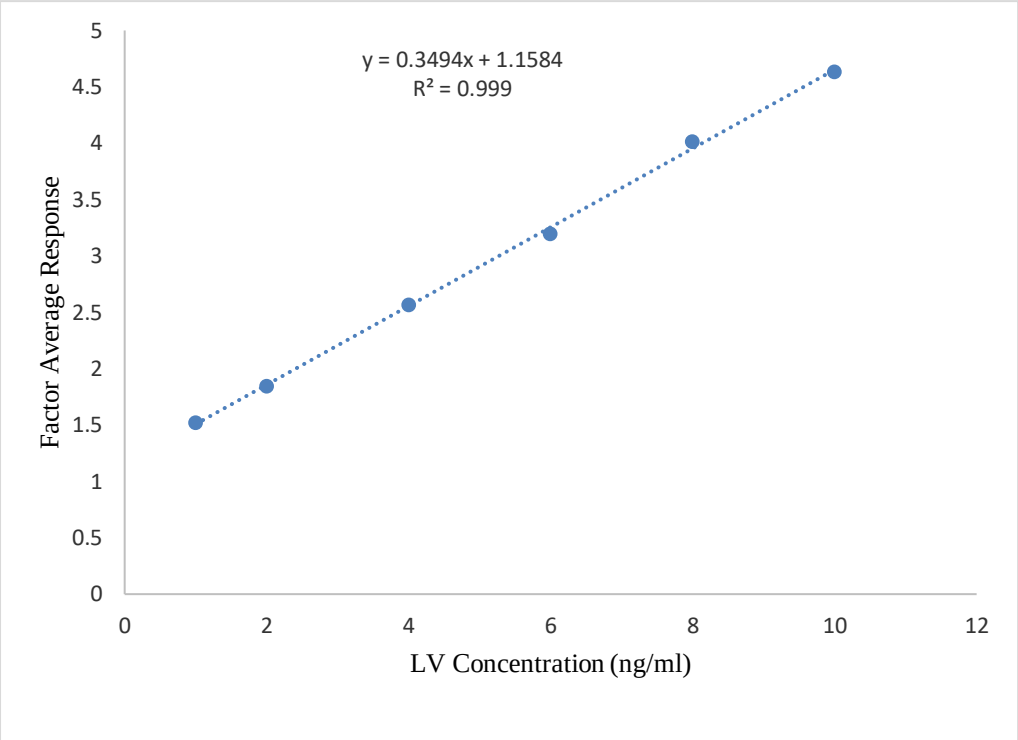

Fig. 55: Calibration curve of Lovastatin LV standard

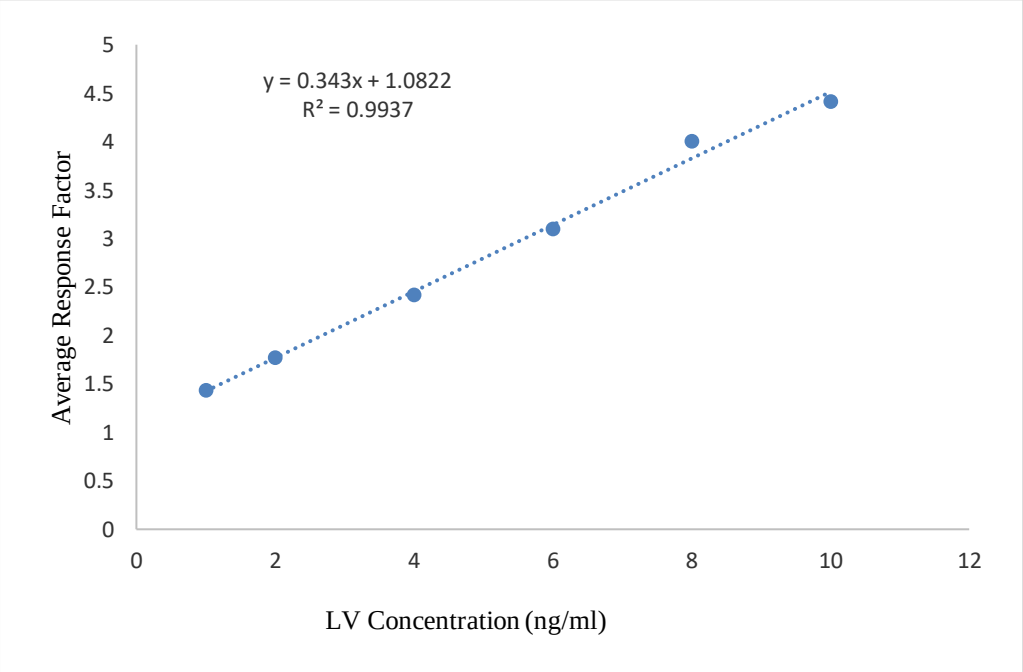

Fig. 56: Calibration curve of LV in Spiked Plasma

7.12.3 Linearity of OA by LC-MS/MS system

Linearity was evaluated for oleanolic acid within the concentration range of 100–600 ng/mL. Each sample was analyzed in triplicate, and the corresponding peak areas were recorded. The response factor was determined by calculating the ratio of the drug's peak area to that of the internal standard. Table 53 and 54 display the response factors for the OA standard and spiked plasma samples, respectively. A calibration curve was generated by plotting the response factors against their respective concentrations. Fig. 57 and 58 illustrate the calibration curves for the OA standard and spiked plasma samples. The calibration curves for OA exhibited strong linearity within the 100–600 ng/mL concentration range. Both the OA standard and spiked plasma calibration curves followed a linear equation, confirming a proportional relationship between concentration and response. The coefficient of determination (R^2) values reflected high accuracy and precision in the quantification process.

Table 53: Response factor for linearity of OA standard

Replica 1				
OA Concentration (ng/mL)	BN Concentration (ng/mL)	OA Area	BN Area	OA Response Factor
100	50	598231	271855	2.201
200	50	1048372	294156	3.564
300	50	1532453	278859	5.495
400	50	2067532	265186	7.797
500	50	2542916	261456	9.726
600	50	3068494	265684	11.549
Replica 2				
OA Concentration (ng/mL)	BN Concentration (ng/mL)	OA Area	BN Area	OA Response Factor
100	50	591743	272127	2.175
200	50	1094698	294450	3.718
300	50	1616842	278301	5.810
400	50	2100480	265451	7.913
500	50	2500990	261717	9.556
600	50	3182566	265153	12.003
Replica 3				
OA Concentration (ng/mL)	BN Concentration (ng/mL)	OA Area	BN Area	OA Response Factor
100	50	605549	271311	2.232
200	50	1123470	293568	3.827
300	50	1606127	279138	5.754
400	50	2087570	264656	7.888
500	50	2580627	260933	9.890
600	50	3035999	265950	11.416
Average Response Factor				
OA Concentration (ng/mL)	Response Factor 1	Response Factor 2	Response Factor 3	Average Response Factor
100	2.201	2.175	2.232	2.203
200	3.564	3.718	3.827	3.703
300	5.495	5.810	5.754	5.653
400	7.797	7.913	7.888	7.866
500	9.726	9.556	9.890	9.724
600	11.549	12.003	11.416	11.656

Table 54: Response factor for linearity of OA in Spiked Plasma

Replica 1				
OA Concentration (ng/mL)	BN Concentration (ng/mL)	OA Area	BN Area	OA Response Factor
100	50	583778	264794	2.205
200	50	955125	286802	3.330
300	50	1516167	261616	5.795
400	50	1925628	258556	7.448
500	50	2475963	254920	9.713
600	50	2963538	268783	11.026
Replica 2				
OA Concentration (ng/mL)	BN Concentration (ng/mL)	OA Area	BN Area	OA Response Factor
100	50	572285	265059	2.159
200	50	1046625	286228	3.657
300	50	1554904	271344	5.730
400	50	2042662	258297	7.908
500	50	2580819	274410	9.405
600	50	2998704	268524	11.167
Replica 3				
OA Concentration (ng/mL)	BN Concentration (ng/mL)	OA Area	BN Area	OA Response Factor
100	50	573515	264529	2.168
200	50	1025002	286515	3.577
300	50	1467012	271888	5.396
400	50	2037325	258039	7.895
500	50	2505252	254665	9.837
600	50	2912353	259042	11.243
Average Response Factor				
OA Concentration (ng/mL)	Response Factor 1	Response Factor 2	Response Factor 3	Average Response Factor
100	2.205	2.159	2.168	2.177
200	3.330	3.657	3.577	3.521
300	5.795	5.730	5.396	5.640
400	7.448	7.908	7.895	7.750
500	9.713	9.405	9.837	9.652
600	11.026	11.167	11.243	11.145

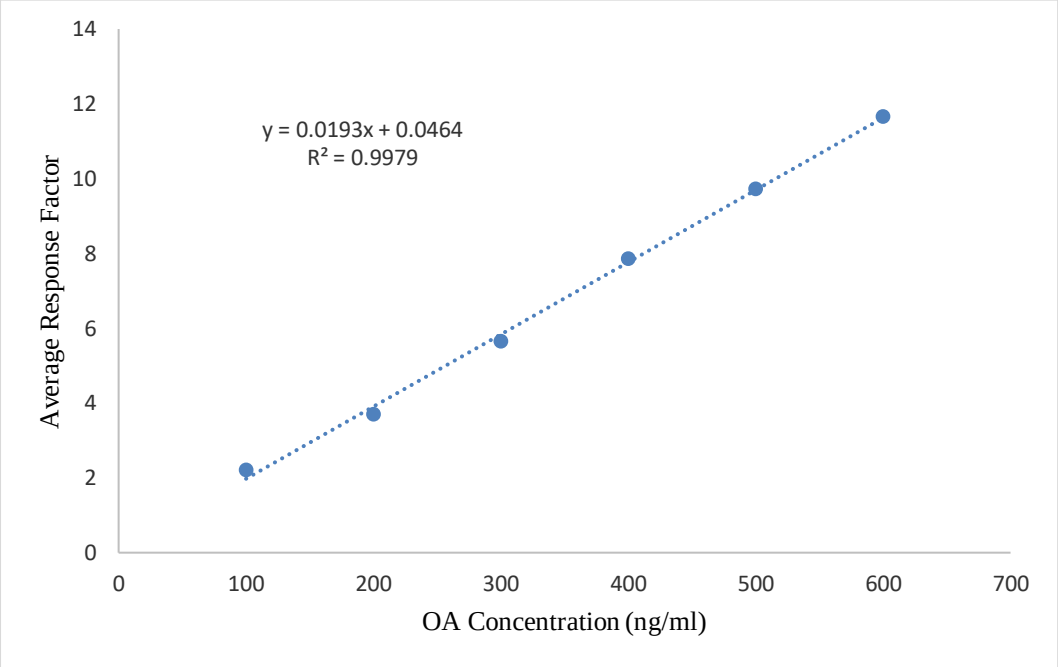

Fig. 57: Calibration curve of OA standard

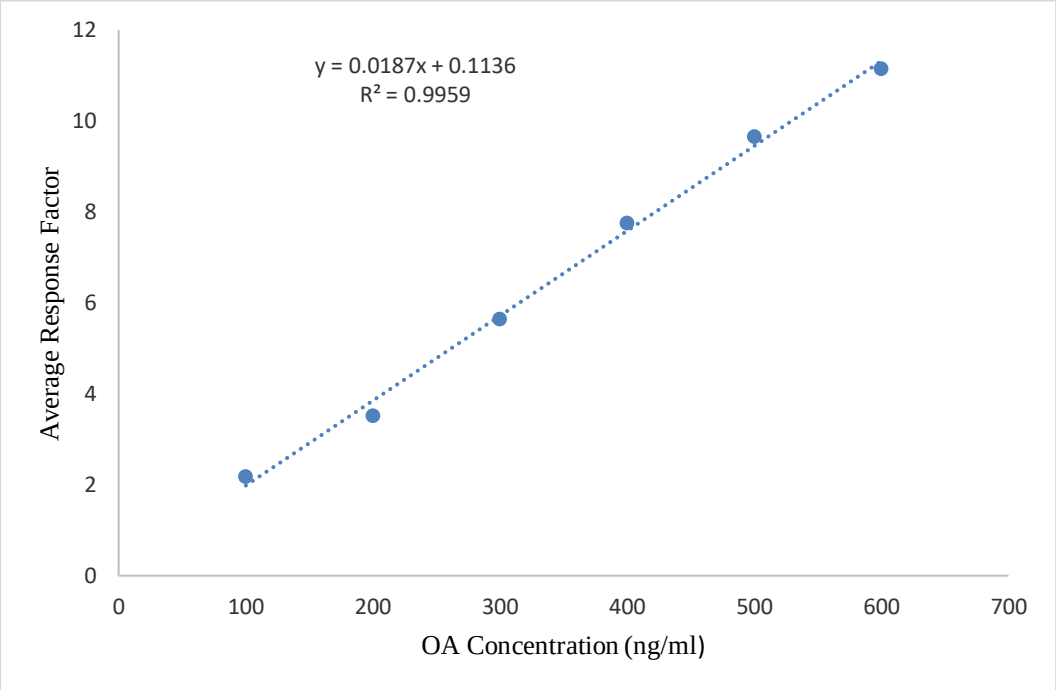

Fig. 58: Calibration curve of OA in Spiked Plasma

7.12.4 Pharmacokinetic Analysis

7.12.4.1 Plasma Concentrations of LV

The comparative analysis of Lovastatin plasma concentrations from core and coat tablets and bilayer tablets indicates similar pharmacokinetic profiles (Table 55). Both formulations exhibited peak concentrations at 0.5 hours, followed by a gradual decline over time. Minor variations in plasma levels were observed, but overall, the differences were not significant. By the 10th hour, both formulations reached nearly identical concentrations, suggesting comparable drug release and absorption patterns. These findings indicate that both formulations offer similar bioavailability. Therefore, either formulation could be appropriate for therapeutic use, depending on formulation preferences and manufacturing considerations. The pharmacokinetic analysis of Lovastatin from core and coat tablets and bilayer tablets (Fig. 59) revealed similar absorption and elimination characteristics. Both formulations reached C_{max} at 0.5 hours (T_{max}), indicating rapid drug absorption. The C_{max} values were slightly different, with core and coat tablets (9.246 ng/mL) showing a marginally higher peak concentration compared to bilayer tablets (9.123 ng/mL), suggesting minor variations in bioavailability. However, this difference is minimal and does not significantly impact the overall drug performance. The $t_{1/2}$ values were 2.81 hours for core and coat tablets and 2.87 hours for bilayer tablets, indicating a relatively short elimination time for Lovastatin. This suggests that the drug is metabolized and cleared quickly from the body, requiring frequent dosing to maintain therapeutic levels.

Table 55: LV Plasma Concentration in formulations

Time (h)	Lovastatin from Core and Coat Tablets	Lovastatin from Bilayer Tablets
	Plasma Conc. (ng/mL) ± S.D.	Plasma Conc. (ng/mL) ± S.D.
0	0	0
0.25	6.238 ± 0.442	6.038 ± 0.476
0.5	9.246 ± 0.319	9.123 ± 0.448
1	8.633 ± 0.654	8.220 ± 0.710
1.5	7.857 ± 0.267	7.533 ± 0.388
2	7.202 ± 0.272	6.904 ± 0.474
3	5.577 ± 0.545	5.977 ± 0.515
4	4.693 ± 0.436	4.864 ± 0.389
6	3.310 ± 0.508	3.327 ± 0.739
10	1.942 ± 0.181	1.939 ± 0.304

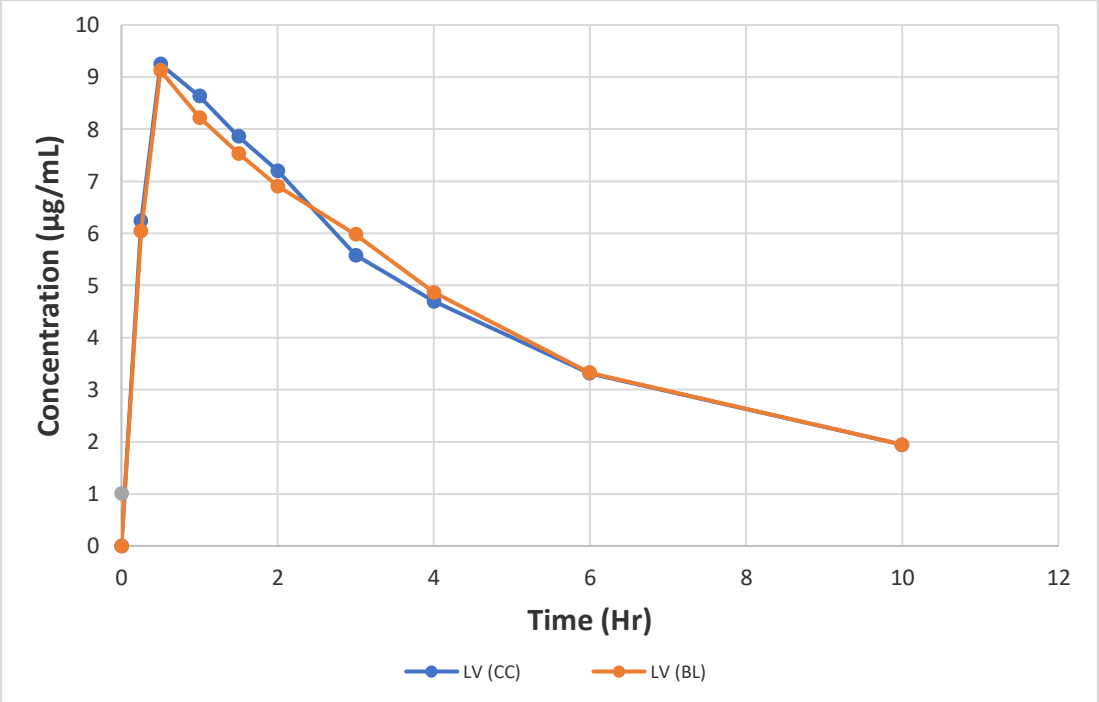

Fig. 59: Pharmacokinetic profile of LV

7.12.4.2 Plasma Concentrations of OA

The plasma concentration profile of Oleanolic Acid (OA) from core and coat tablets and bilayer tablets exhibited similar pharmacokinetic behaviour over the study period (Table 56). Both formulations demonstrated a gradual increase in plasma concentration, at 6 hours, followed by gradually decline, indicating sustained drug release from both formulations. At 24 hours, the concentration remained above 250 ng/mL, confirming prolonged systemic availability. These results indicate that both formulations provide effective and sustained drug release, supporting less frequent dosing and prolonged therapeutic effects. The pharmacokinetic analysis of oleanolic acid from core and coat tablets and bilayer tablets revealed similar drug absorption and elimination patterns (Fig. 60). Both formulations achieved a T_{max} of 6 hours (Table 56), indicating that they reach peak plasma concentration at the same rate. The C_{max} for bilayer tablets (501.128 ng/mL) was slightly higher than that of core and coat tablets (482.635 ng/mL) (Table 57), suggesting a marginally greater bioavailability for the bilayer formulation. However, this difference is minor and does not significantly impact overall drug effectiveness. The $t_{1/2}$ values for core and coat tablets (18.9 hours) and bilayer tablets (18.3 hours) indicate sustained drug release, ensuring prolonged therapeutic effects. The extended half-life suggests that both formulations support less frequent dosing, which could improve patient adherence to treatment. Since both formulations exhibit a similar decline in plasma concentration over time, their elimination rates appear to be consistent. The slight variations in C_{max} could be attributed to differences in formulation composition and drug release mechanisms. Overall, the results confirm that both formulations provide efficient and sustained drug release, making them appropriate for long-term therapeutic use. The comparable pharmacokinetic profiles indicate that either formulation can be used interchangeably, depending on patient preference or manufacturing considerations.

Table 56: OA Plasma Concentration in formulations

Time (h)	Oleanolic Acid from Core and Coat Tablets	Oleanolic Acid from Bilayer Tablets
	Plasma Conc. (ng/mL) ± S.D.	Plasma Conc. (ng/mL) ± S.D.
0	0	0
0.25	107.799 ± 8.055	108.653 ± 6.566
0.5	146.445 ± 5.995	150.002 ± 6.171
1	170.929 ± 8.412	176.965 ± 10.586
1.5	208.41 ± 16.365	211.132 ± 14.819
2	286.407 ± 17.328	275.701 ± 19.137
3	368.62 ± 17.672	383.536 ± 24.472
4	425.323 ± 12.355	441.451 ± 16.606
6	482.635 ± 20.173	501.128 ± 26.320
10	402.82 ± 35.875	405.877 ± 23.616
16	315.713 ± 13.095	316.26 ± 24.380
24	250.463 ± 9.617	251.421 ± 26.272
48	125.082 ± 6.821	125.184 ± 8.916

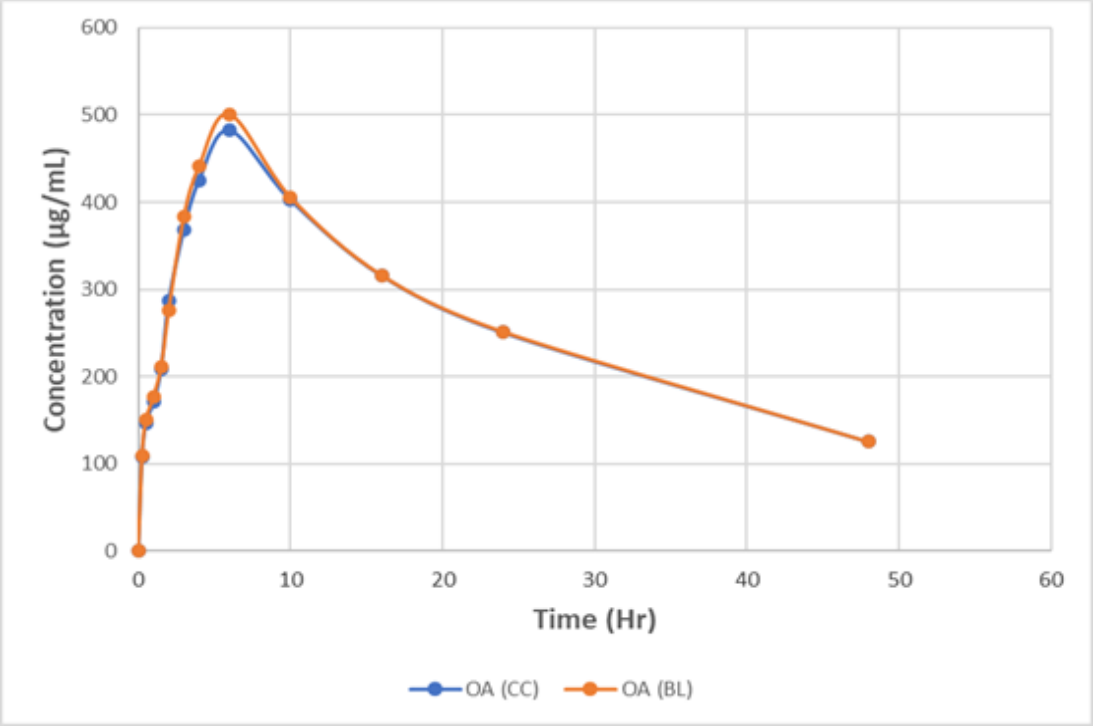

Fig. 60: Pharmacokinetic profile of OA

Table 57: Pharmacokinetic parameters of Bilayer tablet and core and coat tablet

PK parameter	Unit	Optimized core and coat tablet		Optimized Bilayer tablet	
		LV	OA	LV	OA
$t_{1/2}$	h	2.81	18.9	2.87	18.3
T_{max}	h	0.5	6	0.5	6
C_{max}	ng/mL	9.24	482.63	9.12	501.12
$AUC_{(t48-\infty)}$	ng/mL × h	11.47	3983.50	11.75	3912.00
$AUC_{(0-48)}$		44.71	12666.59	44.74	12794.69
K_{el}	h^{-1}	0.16	0.03	0.16	0.03

8. CONCLUSION:

The present investigation was undertaken to design and develop bilayer tablets and core-and-coat tablets containing lovastatin and oleanolic acid with the objective of achieving an immediate lipid-lowering effect followed by sustained antihyperlipidemic action. A simple, specific, accurate, and precise RP-HPLC method for the simultaneous estimation of lovastatin and oleanolic acid was successfully developed and validated in accordance with ICH guidelines, confirming its suitability for routine analysis and formulation evaluation.

A systematic 3^2 factorial design approach was employed to optimize formulation variables. In the immediate-release layer, sodium starch glycolate and croscarmellose sodium significantly influenced drug release at 30 minutes and disintegration time, whereas in the sustained-release layer, HPMC K100 and ethyl cellulose governed the controlled release behavior of oleanolic acid. Statistical optimization and response surface analysis enabled identification of optimized formulations that achieved rapid lovastatin release and prolonged oleanolic acid release up to 12 hours, demonstrating effective modulation of release kinetics.

The optimized bilayer and core-and-coat tablets exhibited reproducible in-vitro dissolution profiles with an initial burst release for lovastatin followed by sustained delivery of oleanolic acid. Stability studies confirmed the physicochemical integrity and dissolution consistency of the optimized batch over six months, indicating formulation robustness.

Importantly, *in vivo* pharmacodynamic evaluation in a rabbit model demonstrated significant reductions in total cholesterol, triglycerides, LDL cholesterol, and body weight, along with increased HDL cholesterol, when compared with disease control animals. The observed in-vivo lipid-lowering effects correlated well with the in-vitro release behavior, confirming effective integration between formulation performance and therapeutic outcomes. Pharmacokinetic and pharmacodynamic findings further indicated enhanced bioavailability and hypolipidemic efficacy of the developed formulations.

Overall, the developed bilayer and core-and-coat tablets of lovastatin and oleanolic acid represent a promising fixed-dose delivery system for the management of hyperlipidemia and associated cardiovascular disorders. The formulation strategy may

potentially reduce dosing frequency, improve patient compliance, and minimize adverse effects. Future studies may focus on long-term clinical evaluation and scale-up feasibility to establish translational applicability.

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LIST OF ANNEXURES

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RJC

RASĀYAN *J. Chem.*
Vol. 18 | No. 3 |1423-1430| July-September | 2025
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF LOVASTATIN AND OLEANOLIC ACID IN ANTILIPIDEMIC TABLETS

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ABSTRACT

In the modern era, rapid high-performance liquid chromatography (HPLC) is the most effective system for the separation of active pharmaceutical ingredients for pharmaceutical analysis due to its extremely effective separation with high sensitivity and accuracy. Establishing and validating an analytical technique using HPLC is essential for upholding the quality of products, as it gives data on accuracy, linearity, precision, and detection. This study focused on developing and validating an HPLC technique for effective evaluation of lovastatin (LV) and oleanolic acid (OA) in core and coat antilipidemic tablets formulated. RP-HPLC process for concurrent assessment of lovastatin also oleanolic acid was performed by means of a Jasco HPLC scheme in addition SunQSil C18 4.6 × 250 mm column (5 µm). The mobile phase utilized for the proposed method was Methanol as well as Water (90:10 v/v), the mobile phase was adjusted to pH 3 using orthophosphoric acid, with a flow rate of 1 mL per min, also finding of both drugs was performed at 210 nm. The validation of the proposed method was carried out in accordance with ICH Q2 (R1) guidelines. Retention time of Lovastatin, in addition to Oleanolic acid, was identified as 4.41 also 6.43 min, respectively. Linearity for Lovastatin also Oleanolic acid was measured in the interval of 2-12 µg/mL with mean percent recovery of 99.99% and 99.82%, respectively. The method demonstrated satisfactory precision, as the percentage relative standard deviation values for both intra- and inter-day precision were below 2%. The proposed simultaneous estimation technique presented exceptional precision, accuracy, linearity, as well as robustness. This simple technique may effectively be applied for the simultaneous estimation of LV as well as OA in therapeutic dosage form. This contributes significantly to ensuring the safety and efficacy of antilipidemic drugs, supporting broader public health goals.

Keywords: Lovastatin, Oleanolic Acid, RP-HPLC, Simultaneous Estimation, Validation, Antilipidemic Tablets.
RASĀYAN *J. Chem.*, Vol. 18, No. 3, 2025

INTRODUCTION

With over 18 million deaths each year, cardiovascular diseases are the leading cause of mortality worldwide. Lipid-lowering agents, such as statins and natural compounds, play a vital role in prevention and management. Their therapeutic success relies heavily on accurate dosing and purity, highlighting the need for reliable analytical methods to ensure quality control and patient safety.[1] This study supports Sustainable Development Goal 3, which focuses on promoting health and well-being for people of all ages. By developing and validating a robust RP-HPLC method for the simultaneous estimation of lovastatin and oleanolic acid, this research contributes to the safe, effective, and affordable use of essential medicines in the fight against non-communicable diseases. Lovastatin (LV), a natural statin, is a secondary metabolite produced by Aspergillus terreus and Monascus ruber in the 1970s.[2] Lovastatin (LV) is chemically "(1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a hexahydronaphthalen-1-yl (2S)-2-methylbutanoate" as shown in Fig.-1a. LV decreases lipid levels by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase; thus, it acts as a potent cholesterol-lowering drug. The[3] Key regulatory steps in cholesterol biosynthesis are catalyzed by this enzyme in the liver (Mevalonate pathway). Also, LV reduces low-density lipoprotein and triglyceride levels and raises the level of high-density lipoprotein.[4] It has been confirmed that in its therapeutic use, LV is also valuable in the treatment of vital diseases such as arterial sclerosis, septicemia, peripheral arterial disease, strokes, and bone fractures.[5] As a biologically active compound, oleanolic acid (OA) is a pentacyclic triterpenoid of the oleanane type, generally present in nearly 200 different varieties of plants.[6]

http://doi.org/10.31788/RJC.2025.1839268

As per the research, oleanolic acid has various pharmacological actions like hepatoprotective, antioxidation, lipid balance, anticancer, and anti-inflammatory.[7] Chemically Oleanolic acid is "(4aS,6aS,6bR,8aR,10S,12aR,12bR,14bS)-10-hydroxy-2,2,6a,6b,9,9,12a-heptamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-4a-carboxylic acid" (Fig.-1b).[8] OA is a specific inhibitor of Intestinal acyl-CoA:cholesterolacyl transferase (ACAT), is intracellular enzyme involved in the cholesterol absorption process from the small intestine and converts into cholesteryl esters, which are transferred to the liver. The accumulation of cholesteryl ester in macrophages leads to the formation of foamy cells and which is nothing but the hallmark of the initial stage of atherosclerosis.[9,10] Cholesterol absorption at the small intestine is reduced by OA, also it reduces plasma cholesterol and triglyceride levels by retarding absorption and retention of metabolic fatty acid, decreases VLDL synthesis in liver that can block deposition of lipids in blood vessels, thus reducing the possibility of heart attacks.[11] Elevated levels of cholesterol and triglycerides raise the risk of cardiac diseases, such as cardiovascular disease, as well as myocardial infarction. By inhibiting ACAT, oleanolic acid acts as an effective and safe agent in the treatment of diseases caused due to increased levels of lipids.[12] Co-administration of LV and OA in fixed doses, respectively, reduces the cholesterol levels that are synthesized by the liver and absorbed from the diet.[13,14]

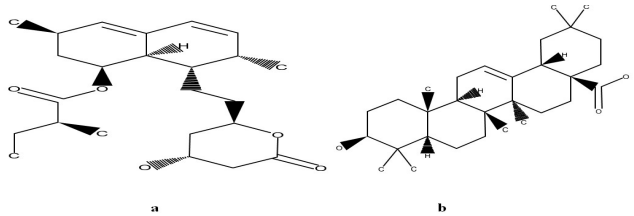

Fig.-1: Chemical Structures of (a) Lovastatin, (b) Oleanolic Acid

This study aims to develop and validate a straightforward, reliable, and precise RP-HPLC method for the concurrent estimation of lovastatin and oleanolic acid in combined antilipidemic tablet formulations. Despite their pharmacological significance, no validated method currently exists for their simultaneous quantification in a single dosage form, highlighting a crucial gap in analytical methodology for combination therapies.

EXPERIMENTAL

Materials and Methods

Pharmaceutically pure gift sample of Lovastatin was provided by Lupin Pharmaceutical Ltd, Mumbai, and Oleanolic acid was procured from Shri Samartha Enterprises, Pune. Chemicals, as well as solvents used, were of analytical and HPLC grade. The HPLC setup included a mobile phase delivery pump (PU 2080 Plus Intelligent), a JASCO UV-2075 UV-VIS detector, a Rheodyne sample injection port with a 20 µl loop, also Borwin chromatography software (Version 1.50). A C-18 reverse-phase column (SunQSil250 × 4.6, 5 µm) was used for evaluation and separation of Lovastatin (LV) and Oleanolic acid (OA) in LV-OA mixture. For the proposed method, the mobile phase comprised methanol in addition to water (90:10 v/v), with the adjustment of pH to 3. The system operated with a maintained flow rate of 1 mL/min also the finding of both drugs occurred at 210 nm, with the column temperature set to 30°C.

Preparations of Standard Stock Solutions

A Lovastatin stock solution (1000 µg/mL) was prepared by dissolving 10 mg of the drug in 10 mL of methanol, yielding a final concentration of 1000 µg per mL. Similarly, to obtain a final concentration of 1000 µg per mL, 10 mL of methanol was used to dissolve 10 mg of Oleanolic acid to prepare a standard stock solution (1000 µg per mL). A 1 mL aliquot of the prepared standard stock solution was transferred and diluted with methanol to a total volume of 10 mL, resulting in final concentrations of 100 µg/mL for both Oleanolic acid and Lovastatin.

Preparation of Working Stock Solution

For the construction of a linearity curve, starting with the standard stock solution (100 µg/mL), the stock solutions were formulated by adding a suitable volume of mobile phase to form a linearity range of 2-12 µg per mL for Oleanolic acid as well as Lovastatin, each.

Analytical Discussion

The analytical method was designed and validated in line with ICH guidelines, evaluating critical parameters including specificity, system suitability, linearity, accuracy, precision, robustness, as well as the limits of detection (LOD) and quantification (LOQ). Specificity was confirmed by injecting standard, sample, placebo, and blank solutions to ensure clear separation of analyte peaks from other matrix components. System suitability tests demonstrated that the entire analytical setup, including equipment and reagents, was appropriate for the analysis. The linearity of the method was assessed within the concentration range of 2 to 12 µg/mL for both LV and OA, showing a strong correlation between concentration and response. Accuracy was assessed using spike recovery at 50%, 100%, and 150% levels of a 4 µg/mL sample, with results expressed as mean %RSD. Precision studies included intraday and interday analyses of samples at 4, 6, and 8 µg/mL, confirming the method's reproducibility and repeatability. Robustness was tested by varying flow rate, mobile phase composition, and detection wavelength, with minimal impact on recovery, peak area, and retention time. The limit of detection (LOD) and limit of quantitation (LOQ) were determined using the signal-to-noise ratio, applying the equation $A = K(\sigma/S)$, where K is 3.3 for LOD and 10 for LOQ, and σ represents the standard deviation and S is the slope of the calibration curve. Overall, the method proved reliable and suitable for routine analysis of the drug combination.[15]

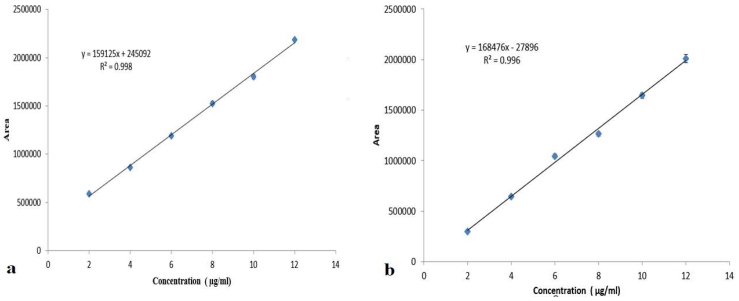

Fig.-2: Calibration Curve of a) Lovastatin, b) Oleanolic Acid

RESULTS AND DISCUSSION

Simultaneous Estimation of LV and OA: Selection of Mobile Phase

Various mobile phases were tested for the establishment of a technique for the simultaneous estimation of LV as well as OA to find the best solution for the differentiation of both active pharmaceutical ingredients by altering mobile phase composition (Fig.-3).

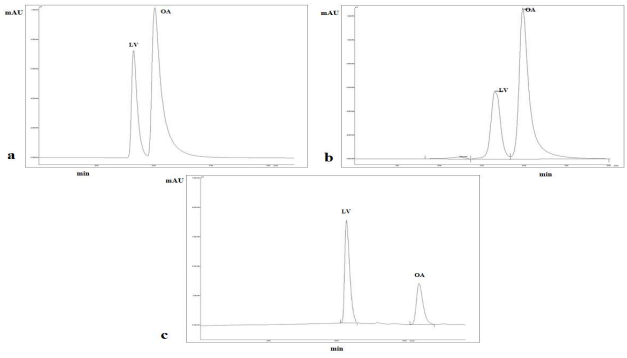

Fig.-3: Chromatogram of a mixture of (a) Lovastatin and Oleanolic Acid with ACN: MEOH (50:50 v/v), (b) Lovastatin as well as Oleanolic Acid with ACN: MEOH (20:80 v/v), (c) Lovastatin as well as Oleanolic Acid MeOH: Water pH 3 Adjusted with OPA (90:10 v/v)

The Acetonitrile (ACN): Methanol (50:50 v/v) was initially used, but a considerable number of theoretical plates were not observed in the result. The methanol was subsequently tried with a high proportion. Methanol: Water in a 90:10 v/v, was adjusted to a pH of 3 using Ortho Phosphoric Acid (OPA), produced a sufficient number of theoretical plates and well-defined peaks. For LV and OA, the retention times observed were 4.41 min., in addition to 6.43 min., correspondingly (Fig.-3). The

optimized mobile phase, methanol: water (90:10 v/v, pH 3 adjusted with OPA), achieved well-defined peaks with retention times of 4.41 minutes for LV and 6.43 minutes for OA.

Specificity

It was estimated by inserting a placebo as well as a blank solution into the analytical system and analyzing the peaks observed in the sample solution with the standard solution. There was no interference between the two peaks. The detected peaks in the sample solution aligned with the standard peaks of Oleanolic acid and Lovastatin, confirming the specificity of the method. The method demonstrated specificity, with clear alignment of sample and standard peaks without interference.

System Suitability

The assessment of system suitability performance involved the examination of factors, including theoretical plates, retention time, asymmetric factor, as well as resolution. The results indicated satisfactory performance of the method within the system (Table-1) as the column efficiency determined was found to be more than 2000 USP plate count; the asymmetric factor for the same peak is not more than 2.0. The method demonstrated good system suitability with high column efficiency and acceptable asymmetry, ensuring reliable performance.

Table-1: System Suitability Parameters for Oleanolic Acid and Lovastatin

Drugs	Concentration µg/ml	RT (min)	Area	Plates	Asymmetry	Resolution
Lovastatin	4	4.417	865950.68	2871.08	1.19	--
Oleanolic acid	4	6.433	648344.75	2970.43	1.32	6.26

Linearity

The constructed calibration curves displayed linearity in the concentration interval of 2-12µg/ml for Oleanolic acid as well as Lovastatin, each (Tables-2 and 3). The correlation coefficient (R^2) values for LV along with OA were observed to be 0.998 and 0.996, respectively (Fig.-2). Also, the technique was verified with the help of ANOVA analysis, and it demonstrates linear regression with no deviation from linearity ($P < 0.05$). The method exhibited strong linearity for both Oleanolic acid and Lovastatin, with high correlation coefficients and no significant deviations confirmed by ANOVA.

Table-2: Linearity Study of Oleanolic Acid

Conc.				Area			Average	SD	%
µg/mL	1	2	3	4	5	6			RSD
2	297455.80	297757.76	297656.80	291964.90	297581.10	297667.88	296680.70	2312.47	0.77
4	648344.75	649876.66	642343.65	634563.66	633456.65	662321.98	645151.22	10808.37	1.67
6	1051637.30	1043454.76	1054538.00	1004245.65	1054325.80	1066893.98	1045849.24	20727.04	1.98
8	1262222.93	1287678.76	1276568.90	1235434.78	1243543.99	1278978.66	1264071.33	20883.33	1.65
10	1620619.86	1667876.77	1612345.70	1632147.50	1670543.66	1667493.70	1645171.19	26485.83	1.61
12	1996902.52	1965459.90	2076789.98	1995432.77	1990245.55	2045279.80	2011685.08	40100.69	1.99

Accuracy

It is also expressed as % recovery, which measures the correctness of the analytical method. The mean percentage recovery of LV and OA of all three levels was within the normal limit of 98-102%. It is verified by a % RSD of less than 2% (Table-4). The proposed method demonstrated high accuracy, with mean percentage recovery for LV and OA within 98–102% and a percentage relative standard deviation below 2%.

Table-3: Linearity Study of Lovastatin

Conc.	Area						Average	SD	%
mg/ml	1	2	3	4	5	6			RSD
2	570769.19	595514.63	595630.33	599999.6	585489.96	592885.26	590048.16	10588.47	1.79
4	865950.68	858863.56	855251.627	866262.2	866965.93	858848.66	862023.77	4974.73	0.57
6	1194078.44	1158965.30	1189114.32	1198928.60	1195886.29	1192196.63	1188194.93	14698.39	1.23
8	1504230.82	1521911.48	1507804.29	1533262.25	1518125.66	1562566.26	1524650.13	21272.52	1.39
10	1787201.48	1795998.62	1792302.867	1798189.26	1787846.63	1855847.96	1802897.80	26300.77	1.45
12	2190175.96	2193188.29	2192783.26	2199659.63	2163225.26	2176854.92	2185981.22	13446.39	0.61

Table-4: Accuracy Study for LV and OA

Sample	Recovery Rate	Quantity of Sample (µg per mL)	Added Quantity (µg per mL)	Peak Area	Recovered quantity (µg per mL)	Recovery (%)	Average% Recovery ± % RSD	Mean Recovery (%)
LV	50%	4	2	1195769.23	5.963	99.386	100.089 ± 0.627	99.99
		4	2	1207308.55	6.036	100.592		
		4	2	1204389.62	6.017	100.287		
	100%	4	4	1522347.8	8.012	100.146	99.988 ± 0.179	
		4	4	1520790.99	8.002	100.023		
		4	4	1517860.67	7.983	99.794		
	150%	4	6	1841758.9	10.015	100.152	99.918 ± 0.206	
		4	6	1835587.87	9.976	99.765		
		4	6	1836759.45	9.984	99.838		
OA	50%	4	2	1038349.75	5.998	99.960	100.310 ± 0.693	99.82
		4	2	1037324.77	5.992	99.859		
		4	2	1049976.12	6.067	101.110		
	100%	4	4	1368709.5	7.958	99.481	99.616 ± 0.140	
		4	4	1370435.77	7.969	99.609		
		4	4	1372453.25	7.981	99.759		
	150%	4	6	1719654.76	10.042	100.415	99.555 ± 0.759	
		4	6	1700048.99	9.925	99.252		
		4	6	1695767.8	9.900	98.998		

Precision

For both Lovastatin and Oleanolic acid, RSD results, in the precision studies of intra as well as inter-day, were under 2.0% (Tables-5 and 6). Each drug shows an acceptable range of % RSD values, which indicates the proposed method was satisfactorily précised, having outstanding repeatability and reproducibility.

Table-5: Intra-Day Precision Study for Lovastatin and Oleanolic Acid

Sample	Concentration (µg/ml)	Area	% Recovery	Average % Recovery ± % RSD
LV	4	879284.6	99.450	100.478 ± 1.220
		894486.6	101.834	
		883744.7	100.149	
	6	1207605	100.623	100.178 ± 0.890
		1193523	99.151	
		1208911	100.760	
	8	1554631	102.677	101.569 ± 0.955
		1531639	100.874	
		1535243	101.157	
OA	4	650544	100.673	100.245 ± 0.551
		648976.67	100.441	
		643452.21	99.621	
	6	996204.9	101.310	100.893 ± 0.528
		993819.19	101.074	
		985924.05	100.293	
	8	1309467.94	99.225	99.994 ± 1.125
		1312790.24	99.472	
		1337234.89	101.285	

Table-6: Inter-day Precision Study for Lovastatin and Oleanolic Acid

Sample	Concentration (µg/ml)	Area	% Recovery	Average % Recovery ± % RSD
LV	4	883086.01	100.046	99.937 ± 0.339
		879966.66	99.557	
		884115.96	100.208	

	6	1218557.3	101.768	100.452
		1199641.15	99.791	±
		1199710.78	99.798	1.135
	8	1537564.22	101.339	100.578
		1533129.96	100.991	±
		1512898.71	99.405	1.025
OA	4	651349.07	100.793	100.740
		648967.12	100.439	±
		652677.83	100.990	0.277
	6	1000729	101.758	100.824
		993782.12	101.071	±
		979346.22	99.643	1.070
	8	1314562.36	99.603	99.745
		1309238.89	99.208	±
		1325621.44	100.424	0.622

Robustness

It was studied via small variations for flow rate, mobile phase ratio, and wavelength. In a robustness study, the percentage relative deviation for LV and OA was found to be below 2%, demonstrating the satisfactory robustness of the proposed method. (Tables-7 and 8).

Table-7: Robustness Study for LV

Variables	Value	Lovastatin				
		Concentration (µg per mL)	Average peak area (N=3)	SD	Percent RSD	Mean percent RSD
Mobile phase ratio (A: B) v/v	92:8	4	888304.58	3489.502	0.393	0.570
	90:10	4	880998.2	4965.326	0.564	
	88:12	4	885857.78	6672.563	0.753	
Flow rate (ml/min)	0.95	4	884758.453	3475.567	0.393	0.443
	1	4	885040.653	2174.034	0.246	
	1.05	4	887311.653	6129.858	0.691	
Wavelength (nm)	209	4	883723.790	4890.543	0.553	0.558
	210	4	880002.107	4774.939	0.543	
	211	4	883707.823	5113.734	0.579	

Table-8: Robustness Study for OA

Variables	Value	Oleanolic acid				
		Concentration (µg per mL)	Average peak area (N=3)	SD	percent RSD	Mean percent RSD
Mobile phase ratio (A: B) v/v	92:8	4	641062.177	2616.313	0.408	0.369
	90:10	4	642037.213	2352.470	0.366	
	88:12	4	640069.620	2125.532	0.332	
Flow rate (ml/min)	0.95	4	645340.287	7417.739	1.149	1.026
	1	4	643721.713	9795.305	1.522	
	1.05	4	643628.993	2626.785	0.408	
Wavelength (nm)	209	4	637847.787	2463.044	0.386	0.376
	210	4	644593.493	2573.058	0.399	
	211	4	646319.543	2211.619	0.342	

LOD and LOQ

These parameters were estimated with formulas 3.3 σ/S as well as 10 σ/S, respectively. Here, 'σ' signifies the response's Standard Deviation for the lowest concentration within the range, as well as S denotes the calibration curve's slope. The LOD values for Lovastatin (LV) and Oleanolic acid (OA) were observed to be 0.169 µg per mL as well as 0.250 µg per mL, respectively, with the corresponding LOQ calculated as 0.512 µg per mL for LV and 0.758 µg per mL for OA. (Table-9). The method demonstrated good

sensitivity with low LOD as well as LOQ values for both Lovastatin and Oleanolic acid, ensuring reliable detection at low concentrations.

Table-9: LOD and LOQ of LV and OA

Parameter	LV (μg/mL)	OA (μg/mL)
Limit of Detection	0.169	0.250
Limit of Quantification	0.512	0.758

Assay

Twenty tablets (comprising Core and Coat Tablets prepared in the laboratory), every tablet holding 50 mg of Oleanolic Acid, as well as 10 mg of Lovastatin, were individually balanced and pulverized. A 10 mg equivalent of Oleanolic Acid (Lovastatin 2 milligrams) was placed into a 10 milliliter graduated flask, also subsequently dilutions were made using methanol, reaching a total volume of 10 milliliters. The resultant solution was strained, and subsequent dilutions were performed using methanol to attain 4 µg/ml as the final concentration for Oleanolic Acid as well as 4 µg/ml for Lovastatin separately. This process was repeated 6 times, and each solution was injected under optimized conditions with the recorded area for each drug. The entire procedure was repeated for accuracy, and concentrations along with percent purity were determined using the linear equation (Table-10).

Table-10: Assay of Tablet Formulation

SN	Oleanolic acid			Lovastatin		
	Area of peak	Recovered Quantity (µg/ml)	Recovered percentage	Area of peak	Recovered Quantity (µg/ml)	Recovered percentage
1	654834.90	4.052	101.310	886632.33	4.020	100.491
2	640342.67	3.966	99.159	889962.55	4.024	100.602
3	644723.10	3.992	99.809	882999.67	4.045	101.124
4	640045.87	3.965	99.115	876632.3	4.001	100.033
5	639987.43	3.964	99.107	882563.64	3.961	99.034
6	636342.81	3.943	98.566	884119.41	3.999	99.964
Mean	642712.797	3.98	99.51	4152.35	4.008	100.208
SD	5940.273	3.980	99.511	0.47	0.026	0.651
% RSD	0.924	0.035	0.881	885925.99	0.650	0.650

Present research work developed a simple and validated HPLC method for analyzing Lovastatin (LV) and Oleanolic acid (OA), using a 90:10 methanol-water mobile phase (pH 3 with OPA) and UV detection at 210 nm. The method showed linearity (2-12 µg/mL), precision (% RSD < 2%), and accuracy (99.99% recovery for LV, 99.82% for OA). The LOD in addition to LOQ values were determined to be 0.16 as well as 0.51 µg/mL for Lovastatin (LV), also 0.25 as well as 0.75 µg/mL for Oleanolic acid (OA), correspondingly. The method met ICH Q2 (R1) guidelines, ensuring reliable and precise results. By enabling accurate and cost-effective quality control of pharmaceutical formulations, this work contributes to improving access to safe and effective medications, ultimately enhancing overall public health and well-being.

CONCLUSION

The developed method for the simultaneous estimation of Lovastatin and Oleanolic acid was thoroughly validated for precision, accuracy, robustness, and linearity. The results confirm that the RP-HPLC technique is simple, reliable, accurate, precise, and robust, offering a cost-effective solution for routine quality control. By enabling efficient and dependable analysis of pharmaceutical formulations, this method directly supports SDG 3 - Good Health and Well-Being, by promoting access to safe, effective, and quality-assured medicines. Accurate analytical methods are essential for ensuring the therapeutic efficacy and safety of pharmaceutical products. Thus, the proposed method contributes to strengthening healthcare systems by supporting consistent drug quality monitoring.

ACKNOWLEDGEMENTS

Authors are thankful to Lupin Pharmaceutical Ltd, India, for providing a gift sample of lovastatin.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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[RJC-9268/2025]

ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 18, Issue 4, 2025

Online - 2455-3891
Print - 0974-2441

Research Article

FORMULATION OF SUSTAINED RELEASE CORE AND COAT TABLETS OF LOVASTATIN AND OLEANOLIC ACID: AN *IN VITRO* AND *IN VIVO* ANALYSIS

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Received: 08 February 2025, Revised and Accepted: 19 March 2025

ABSTRACT

Objectives: This research aimed to develop core and coat tablets to enhance the therapeutic efficacy of antilipidemic drugs for treating high cholesterol and triglyceride levels in the blood.

Methods: The core and coat tablets were formulated using a combination of two antilipidemic drugs: Lovastatin (LV) and oleanolic acid (OA). LV was incorporated into an immediate-release (IR) layer with various superdisintegrants, while OA formulated into extended-release layer with hydroxypropyl methylcellulose K100.

Results: The core and coat tablets were evaluated for the release profiles of both layers, and excipients were optimized. The IR layer of LV achieved complete release within 60 min, while the release of OA was sustained for up to 12 h. Among the formulations tested, LV9 (95.23%) for immediate release, and OA1 (97.13%) for sustained release, were found to be most suitable when scaled at the desired drug release up to 30 min and 12 h, respectively. Stability studies demonstrated that the optimized formulation remained stable without any degradation for 6 months. Pharmacokinetic and pharmacodynamic studies conducted in rabbit models examined the effects of LV/OA tablets on lipid profiles and body weight. Obesity was induced in the rabbits through a high-fat diet.

Conclusion: The core and coat LV/OA tablets demonstrated significant efficacy in reducing lipid levels and mitigating weight gain compared to the control group.

Keywords: Core and coat, Oleanolic acid, Lovastatin, Hydroxypropyl methylcellulose, Croscarmellose sodium.

 DOI: http://dx.doi.org/10.22159/ajpcr.2025v18i4.54038. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Lovastatin (LV) is a cholesterol-lowering medication that was initially extracted from the fungus *Aspergillus terreus* [1]. It is approved by the Food and Drug Administration (FDA) for treating and preventing conditions such as coronary heart disease (CHD), hypercholesterolemia, and for adolescents with heterozygous familial hypercholesterolemia. While not FDA-approved, it is sometimes used to reduce cardiac risks associated with noncardiac surgery and to manage noncardioembolic strokes [2]. LV is converted to the active form of beta-hydroxy acid, which works by competitively diminishing the effect of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase [3]. This enzyme is vital for cholesterol production. Additionally, HMG-CoA inhibitors can lower high-sensitivity C-reactive protein levels, enhance endothelial function, reduce inflammation, and inhibit platelet aggregation with anticoagulant properties. Furthermore, a reduction in serum cholesterol levels can lead to increased expression of low-density lipoprotein (LDL) receptors in liver cells, promoting the breakdown of LDL cholesterol [4].

Oleanolic acid (OA) is a biologically active pentacyclic triterpenoid found in nearly 200 different plant species [5]. Research has demonstrated that OA possesses a range of pharmacological properties, including hepatoprotective, antioxidant, lipid-regulating, anticancer, and anti-inflammatory effects. Chemically, OA is defined as (4aS,6aS,6bR,8aR,10S,12aR,12bR,14bS)-10-hydroxy-2,2,6a,6b,9,9,12a-heptamethyl 1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-4a-carboxylic acid [6]. OA specifically inhibits intestinal acyl-CoAacyltransferase (ACAT), an enzyme involved in the metabolism of cholesterol, where it converts cholesterol into cholesteryl esters for transfer to the liver. The accumulation of cholesteryl esters in macrophages can lead to the formation of foamy cells, a key indicator of early atherosclerosis [7]. By reducing cholesterol absorption in the small intestine, OA helps lower plasma cholesterol and triglyceride levels, decreases hepatic production of very-low-density lipoprotein synthesis, and minimizes lipid deposition in blood vessels, lowering chances of cardiac arrest [8]. Elevated cholesterol and triglyceride levels are indicative of CHD, and cardiac infarction [9]. Through its action as an ACAT inhibitor, OA serves as a safe and effective option for managing lipid-related disorders. Co-administering LV and OA can further lower cholesterol levels derived from both hepatic synthesis and dietary absorption [10].

The core and coat tablet is a widely accepted method for achieving controlled drug release with predefined release profiles. This drug delivery system consists of both an immediate and sustained-release layer. In a core and coat tablet, the outer coat layer disintegrates rapidly to provide an initial dose, while the inner core layer delivers the drug gradually over time [11,12]. To prevent both layers from releasing the drug simultaneously, the sustained-release core layer is coated with various polymers that allow for extended-release in the intestine, while the immediate-release (IR) layer dissolves quickly in the stomach. This design can also be applied to create repeat-action tablets [13].

MATERIALS AND METHODS

Materials

A pharmaceutically pure sample of LV was generously supplied by Lupin Pharmaceutical Ltd., Mumbai, while OA was obtained from

Shri Samartha Enterprises in Pune. All other chemicals" and solvents utilized were of analytical and HPLC grade.

Experimental animals

Healthy NewZeland white rabbits of either sex having a weight range of 2–3 kg were divided into various groups, animals were housed individually in stainless steel cages throughout the study and maintained standard laboratory conditions, fed with a normal diet or high-fat diet and water ad libitum. Animals were kept under fasting conditions before the start of the experiment for approximately 18–24 h. Protocol was approved by the Institutional Animal Ethics Committee (IAEC) of AISSMS College of Pharmacy, Pune constituted under the Committee for the Control and Supervision and Experiments on Animals (CPCSEA). Approval No. CPCSEA/IAEC/PT-25/02-2K23.

Methods

Preformulation studies

To confirm the compatibility of the drug and polymer under experimental conditions and ensure no reactions between them, FOURIER transform infrared (FT-IR) spectroscopy was employed. The spectra of samples were obtained using a Shimadzu IRSpirit-X FT-IR spectrometer. About 3 mg of each test sample was mixed with an equal amount of dried KBr and compressed into a disc [14]. The samples were then scanned at 400 cm^{-1}–4000 cm^{-1}.

Development of core layer

The core tablets were formulated to achieve the desired release profile and ensure stability. Each core tablet contained 50 mg of OA along with hydroxypropyl methylcellulose (HPMC K100), ethyl cellulose, magnesium stearate, talc, lactose, and microcrystalline cellulose (MCC) [15]. The formulation process involved precise weighing and thorough mixing of the ingredients using a mortar and pestle to create a good blend. It was then granulated using the wet granulation method and compressed into core tablets with a Cadmac CMD3 tablet punching machine [16]. Batches OA1 to OA9 of the core layer containing OA were formulated using different compositions as outlined in Table 1.

Development of coat layer

All ingredients were carefully weighed in the specified amounts. LV, sodium starch glycolate, croscarmellose sodium, magnesium stearate, talc, and MCC were then combined, and thoroughly grinded with mortar-pestle to create a uniform blend. This blending process is crucial for ensuring even distribution of the ingredients, which is important for the consistent performance of the coating. The uniform powder blend was transferred to a tablet punching machine. The machine was calibrated to compress the blend into tablets, ensuring each tablet had a consistent weight and shape [17,18]. The compression process was closely monitored to maintain consistent tablet quality. Batches LV1 to LV9 of the LV coat layer were formulated according to the compositions listed in Table 2.

In vitro drug release studies

A dissolution study was performed to assess the release profile of OA from the core tablets and LV from the coated tablets. Electrolab, Type II- paddle dissolution apparatus was used with a dissolution medium consisting of 900 mL of 0.1% sodium lauryl sulfate (SLS) solution, maintained at 37±0.5°C, and paddles were rotated at 100 rpm. Sample to be tested is kept in each vessel of the Electrolab Type II paddle apparatus [19]. At specified time intervals, 10 mL aliquots were taken out and sink conditions were maintained with fresh 0.1% SLS solution. The collected samples were analyzed using HPLC to measure concentrations of OA and LV released at each time point [20].

Formulation of core and coat tablets

Core and coat tablets were formulated using OA as the core layer and LV as the coat layer. The optimized formulations, specifically the OA core (OA1) and the IR LV blend (LV9), were selected for this development. The LV blend was divided into two portions, with one half placed in the die to form a powder bed. The optimized core tablet (OA1) was then centered on this bed, and the remaining LV blend was added to cover the core [21,22]. The entire contents were compressed to create the final core and coat tablets.

Table 1: Composition of oleanolic acid core layer tablets

Batch No.	Ingredients (mg)							
	Oleanolic acid	HPMC K100	Ethyl cellulose	Magnesium stearate	Talc	Lactose	Micro crystalline cellulose	Total weight (mg)
OA1	50	10	10	2	3	12.5	12.5	100
OA2	50	10	15	2	3	10	10	100
OA3	50	10	20	2	3	7.5	7.5	100
OA4	50	15	10	2	3	10	10	100
OA5	50	15	15	2	3	7.5	7.5	100
OA6	50	15	20	2	3	5	5	100
OA7	50	20	10	2	3	7.5	7.5	100
OA8	50	20	15	2	3	5	5	100
OA9	50	20	20	2	3	2.5	2.5	100

Table 2: Composition of lovastatin coat layer tablets

Batch No.	Ingredients (mg)							
	Lovastatin	Sodium starch glycolate	Croscarmellose sodium	Magnesium stearate	Talc	Lactose	Micro crystalline cellulose	Total weight (mg)
LV1	10	4	4	4	6	86	86	200
LV2	10	4	8	4	6	84	84	200
LV3	10	4	12	4	6	82	82	200
LV4	10	8	4	4	6	84	84	200
LV5	10	8	8	4	6	82	82	200
LV6	10	8	12	4	6	80	80	200
LV7	10	12	4	4	6	82	82	200
LV8	10	12	8	4	6	80	80	200
LV9	10	12	12	4	6	78	78	200

In vitro drug release studies of core and coat tablets

This study was conducted individually, following the previously outlined procedure [23]. The collected samples were analyzed using HPLC at a wavelength of 210 nm.

Stability studies

Stability testing is crucial to ensure that the quality of manufactured tablets remains consistent over time under varying environmental conditions. This involves evaluating the effects of temperature, humidity, and storage conditions on both physico-chemical characteristics of the tablets [24]. The recommended storage conditions and minimum durations for such tests include long-term testing at 25°C±2°C with 60% RH±5% for 12 months and accelerated testing at 40°C±2°C with 75% RH±5% for 6 months. It is important to confirm that long-term testing will continue to cover the tablet's expected shelf life, as per International Council for Harmonization Q1A guidelines. Accelerated stability tests were conducted for formulations at 40°C±2°C with 75% RH±5% for 6 months [25].

Experimental design

The rabbits were randomly assigned to four groups: Group I (normal control, n=6) was fed a standard diet; Group II (high-fat diet control, n=6) received a high-fat diet containing 1% cholesterol for 4 weeks; Group III (n=6) was given a high-fat diet for 2 weeks, followed by oral administration of LV (0.52 mg/kg body weight) with a standard diet for the next 2 weeks; and Group IV (n=6) was given a high-fat diet for 2 weeks, followed by oral administration of a core-and-coat tablet containing OA in the core (2.65 mg/kg body weight, sustained release) and LV in the coat (0.52 mg/kg body weight, immediate release) with a standard diet for another 2 weeks [26]. The tablets were placed at the pharyngeal site to ensure immediate swallowing by the rabbits. The statistical analysis of the grouped data was conducted using a one-way analysis of variance (ANOVA), followed by a Bonferroni *post hoc* test for multiple comparisons. The results for each group are presented as mean±standard deviation. A statistically significant result was defined as $p<0.05$.

Body weight was recorded throughout the study. Blood samples (0.3 mL) were collected in heparin-treated microtubes (15 µL heparin in 1.5 mL microtubes) via marginal ear vein or jugular vein cannulation at the following time points: 0 (prior to drug administration), 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 10, 16, 24, and 48 h post-dose. The samples were centrifuged at 1500 rpm for 15 min to separate plasma, which was then frozen at −70°C until analysis. Before testing, the samples were thawed to room temperature and analyzed using an API 2000 LC/MS/MS system equipped with a pump (Shimadzu LC 2OADvp) [27].

RESULTS

FT-IR spectroscopy characterizations

To evaluate the compatibility and identify potential interactions between the drugs and excipients, FT-IR spectroscopy was done on both "the physical mixture of the drug" sample and combination with excipients. A FT-IR spectra for LV and OA showed distinct peaks corresponding to their molecular structures. In the case of LV, key peaks were observed at 3015.4 cm-1 (C=C stretching), 3537.2 cm^{-1} (O-H stretching), 1215.1 cm^{-1} (C-O-C stretching), 1054.8 cm^{-1} (C-O stretching), 1379.1 cm^{-1} (C-H bending), and 2963.2 cm^{-1} (C-H stretching). For OA, the characteristic peaks appeared at 1427.37 cm^{-1} (C=C stretching), 3495.13 cm^{-1} (O-H stretching), 1597.11 cm^{-1} (C-O stretching), and 3117.07 cm^{-1} (C-H stretching). The characteristic peak of drug and excipients are shown in Figs. 1 and 2.

Pre-compression characterization of tablets

The granules of LV and OA formulations were assessed for various flow properties, including bulk density (BD) tapped density (TD), angle of repose, Carr's index, and Hausner ratio (HR), with results summarized in Tables 3 and 4.

The BD for OA formulation was ranged between 0.44 and 0.53 g/mL, while the TD ranged from 0.41 to 0.51 g/mL. For the LV formulation, the BD ranged from 0.48 to 0.61 g/mL, and the TD ranged between 0.53 and 0.71 g/mL. The angle of repose values for OA ranged from 16 to 28.40, and for LV, from 18.20 to 26.30, indicating good flowability. The HR for OA formulations ranged between 1.09 and 1.42, while for LV formulations, it was between 1.05 and 1.18. The compressibility index values ranged from 9 to 28.94 for OA formulations and from 6.67 to 18.33 for LV formulations, suggesting that all batches exhibited favorable flow characteristics.

Characterization of the prepared tablets

The core and coat layers of the formulation were tested on the parameters like hardness, friability, and disintegration time (for the coat layer), with results presented in Tables 5 and 6. Hardness for both layers ranged between 3.8 and 5.5 kg/cm^2, while the value for friability was found between 0.1% to 0.24%.

***In vitro* drug release studies**

The disintegration time for the LV coat layer was under 98 s, which is well below the USP limit of 15 min for uncoated IR tablets. Batch LV9

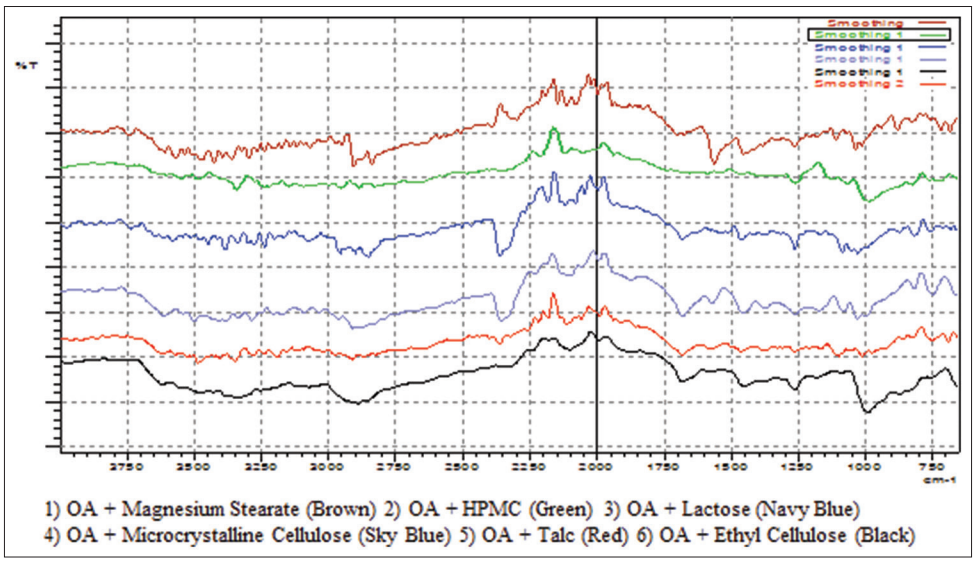

Fig. 1: Drug excipient study of oleanolic acid

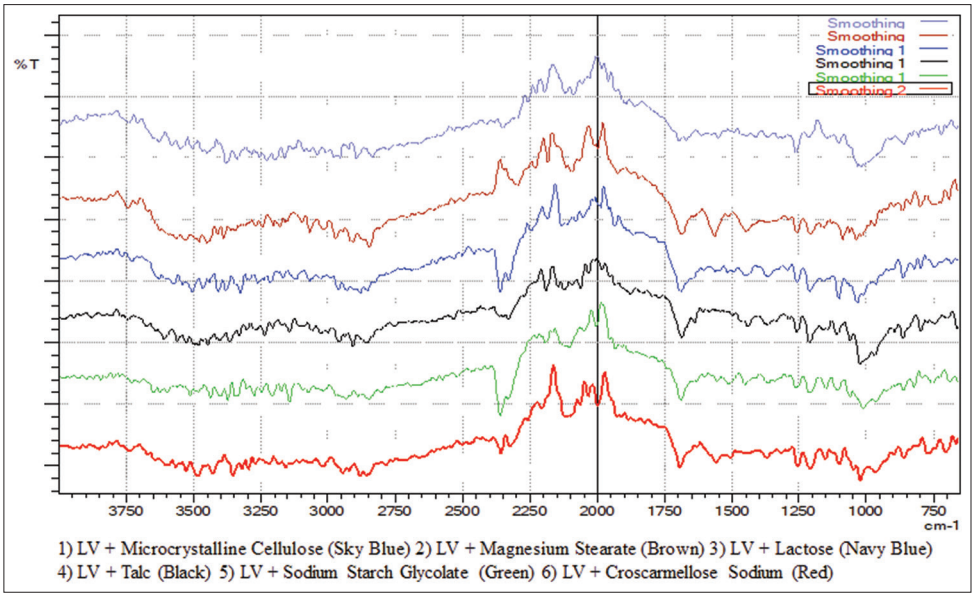

Fig. 2: Drug excipient study of lovastatin

Table 3: Preformulation study of lovastatin coat tablet formulation

Batch No.	BD (g/mL)	TD (g/mL)	HR	CI (%)	Angle of repose (degrees)
LV 1	0.49	0.53	1.15	15.22	21.2
LV 2	0.61	0.71	1.18	18.33	26.3
LV 3	0.57	0.59	1.07	7.27	20.0
LV 4	0.51	0.68	1.13	13.33	21.3
LV 5	0.53	0.64	1.07	6.67	18.2
LV 6	0.54	0.67	1.12	11.67	24.7
LV 7	0.50	0.67	1.12	11.67	24.7
LV 8	0.48	0.54	1.08	8.00	21.4
LV 9	0.59	0.62	1.05	5.08	19.6

BD: Bulk density (g/mL), TD: Tapped density (g/mL), HR: Hausner ratio and CI: Carr's index (%)

Table 4: Preformulation study of oleanolic acid core tablet formulation

Batch no.	BD (g/mL)	TD (g/mL)	HR	CI (%)	Angle of repose (degrees)
OA1	0.53	0.45	1.09	9	16
OA2	0.53	0.45	1.11	11	18
OA3	0.52	0.42	1.13	13	20
OA4	0.49	0.43	1.11	11	17.50
OA5	0.48	0.41	1.11	11	18
OA6	0.52	0.47	1.21	21.50	29
OA7	0.45	0.51	1.42	13.33	21
OA8	0.44	0.49	1.39	22.50	28.40
OA9	0.46	0.49	1.39	28.94	32

BD: Bulk density (g/mL), TD: Tapped density (g/mL), HR: Hausner ratio and CI: Carr's index (%)

demonstrated the fastest disintegration time of 24 s, attributed to the highest concentration of super disintegrants. Drug release studies across various batches (LV1-LV9) with varying ratios of disintegrants revealed that LV9 exhibited the best release profile (Fig. 3), with 95.23% release within 30 min (Table 7). As a result, the LV9 formulation was selected for further development of core and coat tablets.

In the dissolution study for OA core tablets, it was observed that at higher concentrations of HPMC K100 OA release was reduced

Table 5: Evaluation of oleanolic acid core tablets

Batch No.	Hardness (kg/cm^2)	Friability (%)
OA1	3.8	0.23
OA2	4.2	0.18
OA3	4.8	0.24
OA4	4.7	0.21
OA5	5.0	0.19
OA6	4.2	0.20
OA7	4.5	0.18
OA8	4.8	0.21
OA9	4.8	0.23

Table 6: Evaluation of lovastatin coat tablets

Batch No.	Hardness (kg/cm^2)	Friability (%)	DT of coat (sec)
LV1	4.2	0.2	98
LV2	4.6	0.12	75
LV3	5.1	0.16	72
LV4	5.3	0.14	78
LV5	4.8	0.16	72
LV6	4.5	0.15	56
LV7	4.9	0.132	62
LV8	5.3	0.145	36
LV9	5.5	0.1	24

(Fig. 4). The addition of ethyl cellulose formed a more rigid structure in combination with the hydrophilic polymer HPMC K100, effectively retaining the drug within the matrix and slowing diffusion. Among the formulations, batch OA1 demonstrated the highest drug release rate, with 97.13% released over 12 h (Table 8). Based on these findings, OA1 was identified as the optimal formulation for further development of the core and coat tablet system.

Core and coat tablets containing LV in the coat layer and OA in the core layer were formulated using the optimized batches LV9 and OA1. Upon evaluation, all physical parameters were found within acceptable limits. The LV coat layer, formulated from the LV9 batch, had a disintegration time of 24 s. *In vitro* dissolution studies were done with drug release quantified using HPLC. The average percentage of drug release from the core and coat tablets was 96.34% for OA at 12 h and 100.17% for LV at 3 h (Tables 9 and 10).

Table 7: % Release of lovastatin from coat tablets

Batch No.	10 Min	20 Min	30 Min	60 Min	90 Min	120 Min	150 Min	180 Min
LV1	15.25±0.633	32.4±0.560	52.85±0.831	70.2±0.873	90.88±0.758	98.1±0.327	100.3±0.609	99.9±0.530
LV2	21.1±0.509	42.65±0.600	67.23±0.484	88.2±0.892	96.33±0.520	100.65±0.344	101.15±0.714	99.96±0.372
LV3	22.2±0.746	47.95±0.568	73.5±0.735	94.2±0.497	99.15±0.679	100.36±0.702	99.8±0.821	100.25±0.508
LV4	21.66±0.572	44.8±0.615	65.13±0.555	95.65±0.790	98.9±0.556	100.1±0.892	100.55±0.805	101.1±0.494
LV5	20.55±0.321	49.53±0.494	67.89±0.687	94.3±0.937	99.1±0.890	100.25±0.404	100.65±0.834	100.1±0.906
LV6	22.7±0.490	46.1±0.450	72.56±0.640	95.2±0.494	99.25±0.703	99.98±0.375	101.25±0.487	100.9±0.737
LV7	23.5±0.589	51.25±0.478	74.62±0.553	96.23±0.498	99.5±0.633	100.46±0.625	101.1±0.365	100.8±0.636
LV8	28.9±0.592	52.75±0.704	84.28±0.475	97.23±0.420	99.9±0.484	100.15±0.485	99.9±0.749	100.45±0.594
LV9	32.4±0.425	63.7±0.429	95.23±0.393	96.23±0.943	98.99±0.638	100.8±0.869	101.15±0.360	100.5±0.778

Values are in mean±SD (%); (n=6)

Table 8: Drug release profile of oleanolic acid formulations

Time (Hr)	OA1 (%)	OA2 (%)	OA3 (%)	OA4 (%)	OA5 (%)	OA6 (%)	OA7 (%)	OA8 (%)	OA9 (%)
1	16.54±0.701	12.94±0.821	13.73±0.830	22.66±0.591	19.78±0.849	14.12±0.636	21.54±0.630	18.96±0.584	25.82±0.904
2	22.54±0.649	18.23±1.036	19.72±0.581	28.32±0.575	23.43±0.944	21.64±0.802	27.32±0.520	26.22±0.380	32.23±0.597
3	31.10±0.492	25.85±0.564	26.70±0.655	36.55±0.764	30.60±0.564	29.25±0.698	34.95±0.558	32.96±0.995	38.25±0.750
4	49.50±0.910	38.30±0.792	42.44±0.792	45.90±0.610	41.63±0.787	39.84±0.434	43.55±0.670	42.23±0.422	42.44±0.901
6	82.75±0.807	72.66±0.738	73.32±0.957	79.34±0.842	77.96±0.556	75.55±0.659	80.22±0.609	73.88±0.667	75.83±0.299
8	91.65±0.869	79.42±0.422	81.55±0.616	87.64±0.808	86.35±0.732	81.60±0.581	85.25±0.875	80.33±0.936	79.23±0.397
10	95.23±0.946	83.47±0.877	84.32±0.666	88.41±0.391	87.52±0.563	82.62±0.602	85.32±0.564	81.27±0.567	80.23±0.781
12	97.13±0.961	85.14±1.087	86.01±0.823	90.18±0.528	89.27±0.394	84.27±0.821	87.03±0.559	83.96±0.505	82.83±0.395

Values are in mean±SD (%); (n=6)

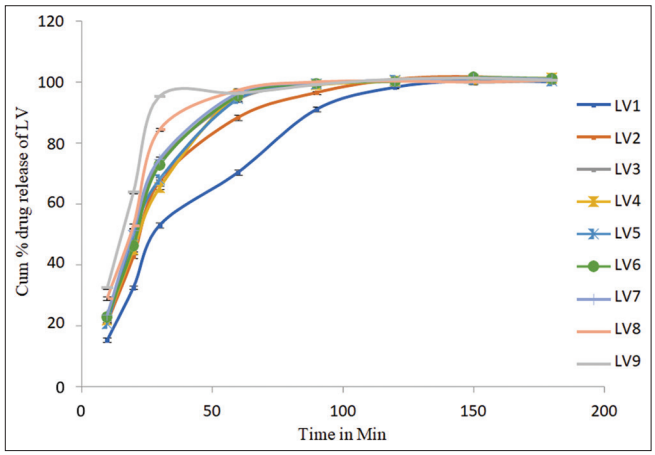

Fig. 3: *In vitro* dissolution studies of lovastatin coat tablets

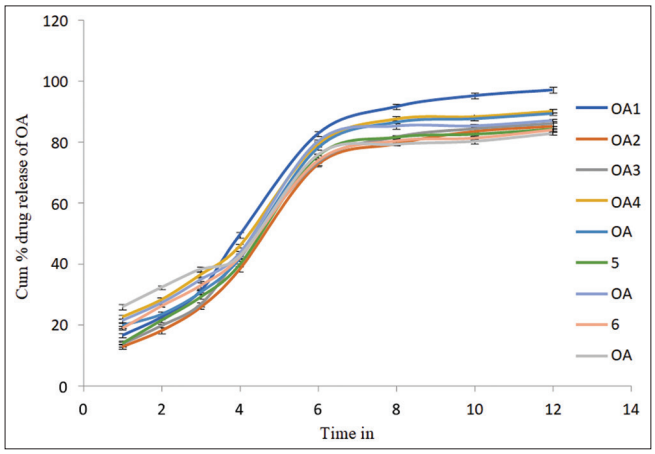

Fig. 4: *In vitro* dissolution studies of oleanolic acid core tablets

Stability studies

Accelerated stability studies conducted over 6 months showed no significant changes in the drug release profile or in other physical parameters, which remained stable throughout the study period as shown in Table 11.

***In vivo* study**

Pharmacodynamic study

Rabbits are widely used as a model to investigate hypercholesterolemia as their lipoprotein profile is comparable to humans. As shown in Table 12, the plasma levels of total cholesterol (TC), LDL, and triglycerides (TG) were increased significantly ($p<0.05$) in rabbits of group II (n=6) after 4 weeks of nutrition with the dietary cholesterol as compared with group I, which was fed with a normal diet. The high-density lipoprotein (HDL) decreased significantly ($p<0.05$) in group II compared to group I after week 4.

After administration of LV API in group III for 2 weeks, the level of TC was decreased as compared to group IV ($p<0.0001$). In group IV after administration of core and coat tablet for 2 weeks, the levels of TG and LDL were decreased ($p<0.0001$). Furthermore, the level of HDL was increased in group IV after 4 weeks compared to the other groups ($p<0.001$). The body weight was increased in group II from the beginning of the experiment till week 4, but in the treated group till week 2, in comparison to the normal control group as presented in Table 13.

Pharmacokinetic study

The plasma concentrations of LV and OA at various time points following the administration of the optimized core and coat tablet are shown in Fig. 5.

Statistical comparisons of the parameters were performed using one-way ANOVA, with a significance level set at $p<0.05$. The pharmacokinetic parameters were calculated based on the plasma concentration-time profiles of both the drugs and the results are summarized in Table 14.

The average peak plasma concentrations (C_{max}) for LV and OA were 9.246±0.319 ng/mL and 482.635±20.173 ng/mL, respectively. The time to reach the maximum plasma concentration (t_{max}) was 30 min for LV and 6 h for OA. The areas under the concentration-time curve (AUC) from 0 to 48 h (AUC_{0-48}) and from 48 h to infinity ($AUC_{t48-\infty}$) for the optimized core and coat tablet were found to be 12666.596 ng h/mL and 3983.503 ng h/mL, respectively. The pharmacokinetic data analysis of the core and coat tablet indicated no significant pharmacokinetic interaction between LV and OA. The combination of these two drugs suggests a potential synergistic effect in lowering lipid levels, which may be beneficial for patients with hypertension and hyperlipidemia.

Table 9: Dissolution study of OA1 of core and coat tablets

Time (min)	Cum. % drug release of oleanolic acid				Standard deviation
	I	II	II	Avg	
60	13.48	16.67	15.39	15.18	1.310
120	18.78	20.95	19.79	19.84	0.886
180	27.33	29.64	28.38	28.45	0.944
240	38.87	40.51	39.84	39.74	0.673
360	64.23	66.39	65.61	65.41	0.893
480	83.1	85.55	84.31	84.32	1.001
600	91.66	93.78	93.32	92.82	0.911
720	97.18	96.12	95.72	96.34	0.616

Table 10: Dissolution study of LV9 of core and coat tablets

Time (Min)	I	II	III	Average	Standard deviation
10	30.53	31.61	33.14	31.76	1.070
20	60.11	61.37	62.21	61.23	0.863
30	90.07	92.31	91.34	91.24	0.917
60	96.17	97.22	98.09	97.16	0.784
90	97.61	99.89	99.08	98.86	0.943
120	101.11	99.91	99.40	100.14	0.716
150	101.73	99.95	100.87	100.85	0.726
180	99.10	101.35	100.06	100.17	0.921

DISCUSSION

The FT-IR spectrum confirmed the absence of interactions between the drugs themselves and between the drugs and excipients, including disintegrants, MCC, and HPMC K100, used in the formulations. The angle of repose values indicated satisfactory flow properties. Disintegration considered the first step in the dissolution process of coated tablets, was analyzed alongside friability and dissolution rate parameters. It was observed that an increase in friability did not significantly impact disintegration time, likely due to the inclusion of super disintegrants. Dissolution studies revealed that the outer coat layer of LV released over 90% of the drug within 30 min, meeting the desired criteria, while the core layer of OA achieved a 97.13% release over 12 h. After 6 months of accelerated stability testing, the physical parameters remained unchanged, and the drug content in the core and coat tablets was within USP specifications. No significant differences were noted in drug release profiles between batches, indicating that the tablets maintained stability throughout the testing period. The combination of LV and OA provides a dual mechanism of action: LV reduces cholesterol production by inhibiting HMG-CoA reductase, while OA decreases dietary cholesterol absorption by inhibiting intestinal ACAT. This release profile is advantageous as it enables rapid LV release from the coat layer for quick action, and sustained OA release from the core layer to maintain steady drug levels in the bloodstream. Formulating LV into IR layer while OA as a SR layer in a core and coat tablet can lower the administration of number of doses and the required API dosage, potentially lowering the risk of adverse effects. The *in vivo* results demonstrate that treatment with LV API effectively reduced TC levels, whereas the administration of the core and coat tablet further decreased TG and LDL levels and improved HDL levels, highlighting its potential therapeutic benefit. Additionally, body

Table 11: Stability study of optimized batch of core and coat tablets

Time	Physical appearance	Weight variation[a]	Hardness (kg/cm^2)[b]	Drug content (%)		Friability (%)
				OA	LV	
Initial	White	305.400±1.586	5.440±0.049	99.511	100.208	0.091
1st Month	White	305.500±1.558	5.380±0.075	99.631	100.059	0.091
3rd Month	White	305.450±1.527	5.500±0.110	99.394	99.800	0.115
6th Month	White	305.300±1.384	5.360±0.080	99.454	99.963	0.124

[a]Values are in mean±SD (mg); (n=6): [b]Values are in mean±SD (kg/cm^2)

Table 12: Changes in lipid profile in rabbits in all groups

Groups	TC	TG	HDL	LDL
Group I (Normal control)	58.67±9.33	50.5±2.93	34.2±4.30	50.17±4.0
Group II (Disease control)	594.7±21.33	214±18.43	13.5±2.64	608.2±57.54
Group III (lovastatin (API)	111.67±19.91	126.17±27.13	19.03±6.30	207.8±44.19
Group IV (core-and-coat tablet)	182.17±42.7	116.63±13.16	31.5±5.01	125.8±23.64

Values are in mean±SD (µg/mL); (n=6); a=p < 0.0001 versus Group III, b=p < 0.0001 versus Group IV; Statistics used: Repeated measures analysis of variance followed by Bonferroni multiple comparison *post hoc* test. TC: Total cholesterol, TG: Triglycerides, LDL: Low-density lipoprotein cholesterol and HDL: High-density lipoprotein cholesterol

Table 13: Changes in body weight (n=6) in rabbits in all groups

Groups	Body weight (gm)			
	1st week	2nd week	2rd week	4th week
Group I (normal control)	2.66±0.13	2.83±0.15	3.03±0.15	3.26±0.19
Group II (disease control)	2.77±0.14	3.16±0.18	3.69±0.12	4.00±0.11
Group III (lovastatin (API)	2.77±0.13	2.86±0.14	2.90±0.19	2.93±0.25
Group IV (core-and-coat tablet)	2.18±0.15	2.95±0.18	3.02±0.25	2.90±0.17

Values are in mean±SD (gm); (n=6)

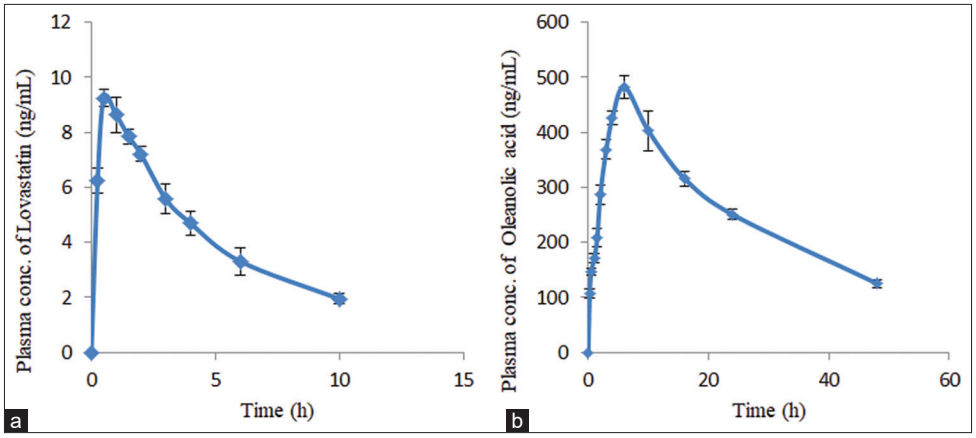

Fig. 5: Mean plasma concentration versus time (mean±SD) profile of optimized core and coat tablet of (a) lovastatin (b) oleanolic acid

Table 14: Pharmacokinetic parameters for lovastatin and oleanolic acid of core and coat tablet

PK parameter[a]	Unit	Optimized core and coat tablet	
		Lovastatin	Oleanolic acid
T_{max}	h	0.5	6
C_{max}	ng/ml	9.246	482.635
$AUC_{(t48-\infty)}$	ng/ml×h	11.478	3983.503
$AUC_{(0-48)}$		44.714	12666.596
K_{el}	h^{-1}	0.1692	0.0314

[a]T_{max}: Time to reach maximum concentration, (C_{max}): Maximum plasma concentration, AUC: Area under plasma concentration-time curve, Kel: Elimination rate constant

weight trends showed an increase in untreated hypercholesterolemic rabbits, whereas the treated groups exhibited moderated weight gain, suggesting a favourable impact of the treatments on metabolic parameters. The pharmacokinetic study of the core and coat tablet formulation revealed improved absorption and bioavailability of LV, along with prolonged therapeutic levels of OA, ensuring a more effective and sustained treatment. These findings support the efficacy of the tested formulations in managing hypercholesterolemia and its associated complications.

CONCLUSION

The combination of immediate and sustained release allows for a dual mode of action, where LV controls cholesterol synthesis and OA inhibits dietary cholesterol absorption. This approach reduces both the frequency of administration and the overall drug dosage, potentially minimizing side effects and improving patient compliance. The *in vivo* study concludes that the core and coat tablet formulation demonstrated significant potential in managing hypercholesterolemia by effectively reducing TC, TG, and LDL levels while increasing HDL levels and moderating body weight in treated rabbits. These findings highlight its therapeutic efficacy compared to LV API and provide a promising approach for cholesterol management. Thus, the core and coat tablet system provides an effective and stable formulation for the combined delivery of LV and OA. The pharmacokinetic study highlighted the rapid absorption of LV, improved oral bioavailability of LV, and sustained therapeutic levels of OA in the bloodstream, which were facilitated by the core and coat tablet formulation approach.

ACKNOWLEDGMENT

The authors are thankful to Lupin Pharmaceutical Ltd, India for providing gift samples of LV.

AUTHOR'S CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing as well as editing, and approved the final draft for publication.

CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

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Invention Title	CORE AND COAT TABLET COMPRISING LOVASTATIN AND OLEANOLIC ACID
Publication Number	2/2025
Publication Date	10/01/2025
Publication Type	INA
Application Number	202411104243
Application Filing Date	30/12/2024
Priority Number	
Priority Country	
Priority Date	
Field Of Invention	CHEMICAL
Classification (IPC)	A61K0009200000, A61K0009500000, A61K0009240000, A61K0008630000, A61P0037060000

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Applicant

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Application Details	
APPLICATION NUMBER	202411104243
APPLICATION TYPE	ORDINARY APPLICATION
DATE OF FILING	30/12/2024
APPLICANT NAME	**LOVELY PROFESSIONAL UNIVERSITY**
TITLE OF INVENTION	CORE AND COAT TABLET COMPRISING LOVASTATIN AND OLEANOLIC ACID
FIELD OF INVENTION	CHEMICAL
E-MAIL (As Per Record)	ashish.iprindia@hotmail.com
ADDITIONAL-EMAIL (As Per Record)	
E-MAIL (UPDATED Online)	
PRIORITY DATE	
REQUEST FOR EXAMINATION DATE	--
PUBLICATION DATE (U/S 11A)	10/01/2025

Application Status	
APPLICATION STATUS	**Awaiting Request for Examination**

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To whomsoever it may concern

This is to certify that the Experimental Procedures conducted for the research project entitled **"Pharmacokinetic and Antihyperlipidemic activity of bilayer and Core & Coat tablets formulation containing Oleanolic Acid and Lovastatin in Rabbits"** of Mr. Satish Vasant Mandave (Ph.D. Student) has been approved by the Institutional Animal Ethics Committee (IAEC) of AISSMS College of Pharmacy, Pune constituted under the Committee for Purpose of Control and Supervision of experiments on animals (CPCSEA), approval No. **CPCSEA/IAEC/PT-25/02-2K23** in the meeting dated 22.05.2023.

Member Secretary

Dr Sachin V. Tembhurne

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Centre for

Research Degree Programmes

LPU/CRDP/PHD/EC/20201005/000682

Dated: 29 Jun 2020

Mandave Satish Vasant
VID: 41800902
Programme Name: Doctor of Philosophy (Pharmaceutics)

Subject: Letter of Candidacy for Ph.D.

Dear Candidate,

We are very pleased to inform you that the Department Doctoral Board has approved your candidacy for the Ph.D. Programme on 29 Jun 2020 by accepting your research proposal entitled: "Development and Evaluation of Modified Release Tablets of Lovastatin and Oleanolic acid"

As a Ph.D. candidate you are required to abide by the conditions, rules and regulations laid down for Ph.D. Programme of the University, and amendments, if any, made from time to time.

We wish you the very best!!

In case you have any query related to your programme, please contact Centre of Research Degree Programmes.

Head

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Note:-This is a computer generated certificate and no signature is required. Please use the reference number generated on this certificate for future conversations.

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CERTIFICATE OF APPRECIATION

Satish Vasant Mandave

of

SVERI's College of Pharmacy, Pandharpur presented a paper as Poster presentation entitled "**RP-HPLC Method Development and Validation for Simultaneous Estimation of Lovastatin and Oleanolic acid**"

during Two Days International Conference on

"**Recent Innovations: A Holistic and Integrated Approach to Pharmaceutical Science**"

held on 23rd to 24th September 2022.

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(Professor, IISc Bangalore, India)

Keynote Speakers

Ashok Ranade
(Project Advisor, Training and Support Services for New High Tech Global Startups, Canada)

Dr. Dhananjay Tambe
(Associate Professor, University of South Alabama, USA)

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This is to certify that ***Mr. Satish Vasant Mandave*** of ***SVERI's College of Pharmacy (Poly.), Pandharpur*** has presented a research paper entitled ***Current Advances in Tablet in Tablet as Drug Delivery System: A Review (Paper ID- 4282)*** in the International Conference on "Advanced Technologies for Societal Applications: Techno-Societal 2022", held at Shri Vithal Education & Research Institute (SVERI), Pandharpur, India during 9th - 10th December 2022.

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This is to certify that Prof./~~Dr./Mr./Ms.~~ Satish Vasant Mandave of SVERI's College of Pharmacy (Poly.), Pandharpur has presented a research paper entitled "Bilayer Tablets: The Dual Action Revolution in Drug Delivery" in the 5th International Conference on "Advanced Technologies for Societal Applications: Techno-Societal 2024", held at Shri Vithal Education & Research Institute (SVERI), Pandharpur, India during 13th-14th December 2024

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