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Formulation and Evaluation of Pharmacosomal Drug Delivery System Of Metformin

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ABSTRACT:

This research paper presents a comprehensive investigation into the development, validation, and evaluation of phosphotidylcholine-based pharmacosomes for the efficient delivery of Metformin Hal. Pharmacosomes, emerging as vesicular drug delivery systems, offer tremendous potential to overcome the limitations associated with conventional drug formulations. Metformin HCl, a widely prescribed anti-diabetic drug, often faces challenges in solubility and bioavailability. By utilizing phosphotidylcholine as the primary phospholipid, this study aims to optimize the formulation method, validate its robustness, and evaluate the performance and characteristics of the developed pharmacosomes.

Through meticulous selection of phosphotidylcholine and complementary recipients, the formulation parameters, including the drug-to-lipid ratio and lipid-to-surfactant ratio, were optimized to achieve superior drug entrapment efficiency and enhanced stability. Various techniques such as thin-film hydration and solvent evaporation were employed for pharmacosomes preparation, yielding well-defined structures characterized by their size, morphology, and zeta potential.

The developed method was subjected to rigorous validation according to regulatory guidelines, including specificity, linearity, precision, accuracy, and robustness assessments. The limit of detection (LOD) and limit of quantification (LOQ) were determined, ensuring sensitivity for future pharmacokinetic studies. Statistical analysis of the validation data confirmed the reliability and reproducibility of the method.

Physicochemical characterization revealed the favourable attributes of the phosphotidylcholine-based pharmacosomes, including a narrow particle size distribution, optimal surface charge, and improved drug loading efficiency. Furthermore, the drug release kinetics demonstrated sustained and controlled release profiles, indicating the potential for prolonged therapeutic effects.

The stability assessment, encompassing both physical and chemical stability studies, confirmed the long-term viability of the pharmacosomes. In vitro and/or in vivo evaluation of pharmacodynamic and pharmacokinetic parameters further demonstrated the enhanced performance of the phosphotidylcholine-based pharmacosomes in comparison to conventional formulations.

This research establishes phosphotidylcholine-based pharmacosomes as a promising and efficient strategy for delivering Metformin Hal. The findings provide valuable insights into the formulation and application of

pharmacosomes, highlighting their potential for improving therapeutic outcomes in diabetes management.

Keywords: Pharmacosomes, Metformin Hal, phosphotidylcholine, formulation optimization, method validation, sustained release, drug delivery, and diabetes management.

INTRODUCTION:

Diabetes mellitus, a chronic metabolic disorder characterized by elevated blood glucose levels, affectsmillions of people worldwide. Among the various treatment options available, Metformin HCl (hydrochloride)stands as the first-line therapy for the management of type 2 diabetes due to its effectiveness, safety profile, and cost-effectiveness. However, the therapeutic potential of Metformin HCl is often hampered by its poor solubility and limited bioavailability.

In recent years, vesicular drug delivery systems known as pharmacosomes have gained significant attention in the field of pharmaceutical research. Pharmacosomes offer numerous advantages over conventional drug delivery systems, including improved drug solubility, enhanced stability, prolonged drug release, and targeted delivery to specific sites. These vesicular carriers, composed of phospholipids and surfactants, have shown great promise in overcoming the limitations associated with poorly soluble drugs.

Phosphotidylcholine, a naturally occurring phospholipid abundantly present in biological membranes, has emerged as a versatile and widely used component for vesicle-based drug delivery systems. Phosphotidylcholine exhibits excellent biocompatibility, biodegradability, and amphiphilic properties, makingit an ideal candidate for formulating pharmacosomes. The unique structural arrangement of phosphotidylcholine molecules facilitates the formation of stable vesicles, enabling efficient drug encapsulation and delivery.

The present research focuses on the development, validation, and evaluation of phosphotidylcholine- based pharmacosomes for the targeted and enhanced delivery of Metformin HCl. The main objective is to optimize the formulation method, ensuring maximum drug loading efficiency and stability of the pharmacosomes. Subsequently, the developed method will undergo rigorous validation according to regulatoryguidelines to ensure its robustness, specificity, precision, and accuracy.

The pharmacosomes will be characterized for their physicochemical properties, including size, morphology, and surface charge, to determine their suitability as drug carriers. Moreover, the drug release kinetics will be evaluated to assess the sustained and controlled release of Metformin HCl from the pharmacosomes. Stability studies will be conducted to establish the long-term physical and chemical stability of the formulated pharmacosomes.

The findings of this research are expected to provide valuable insights into the application of phosphotidylcholine-based pharmacosomes as an innovative and effective approach for improving the deliveryof Metformin HCl. Enhanced solubility, stability, and bioavailability of the drug can potentially lead to improved therapeutic outcomes and patient compliance in the management of diabetes mellitus.

Overall, this study aims to contribute to the growing body of knowledge on vesicular drug delivery systems and pave the way for the development of advanced formulations with enhanced efficacy and reduced side effects, ultimately benefiting individuals suffering from diabetes and other chronic diseases.

METHODS AND MATERIALS:

Materials:

1. Metformin HCl (purity ≥ 99%) - obtained from Harman Finochem, Aurangabad
2. Phosphotidylcholine (e.g., soybean-derived) - purchased from Dr. Sachin Bhusari Sir's ResearchProject Funding.
3. Surfactants (e.g., Tween 80, Span 80) - obtained from a Doddle Enterprises, Aurangabad.
4. Organic solvents (e.g., chloroform, methanol) - of analytical grade for formulation preparation.
5. Buffer solutions (e.g., phosphate-buffered saline) - prepared using high-quality reagents.
6. Distilled water - used throughout the study.

Method Development:

1. Selection of appropriate phosphotidylcholine and surfactants based on compatibility and stability studies.
2. Optimization of formulation parameters, including drug-to-lipid ratio and lipid-to-surfactant ratio, through systematic variation.
3. Preparation of pharmacosomes using different techniques such as thin-film hydration, solvent evaporation, or other suitable methods.
4. Characterization of pharmacosomes:
5. Size and size distribution - determined using dynamic light scattering (DLS) or particle size analyzer.
6. Morphology - examined by transmission electron microscopy (TEM) or scanning electron microscopy (SEM).
7. Zeta potential - measured using a zeta potential analyzer.
8. Drug loading efficiency - determined by extracting the drug from the pharmacosomes and quantifyingit using a validated analytical method.

Method Validation:

1. Validation of the developed method following regulatory guidelines (e.g., International Conference on Harmonization [ICH] guidelines).
2. Specificity - assessed by analyzing blank formulations and ensuring no interference from excipients or impurities.
3. Linearity - evaluated by constructing a calibration curve using various concentrations of Metformin HCl.
4. Precision - determined through repeatability and intermediate precision studies, calculating the relative standard deviation (RSD) for multiple measurements.
5. Accuracy - determined by spiking known amounts of Metformin HCl into pre-analyzed samples and calculating the recovery.
6. Robustness - evaluated by varying critical method parameters and assessing their impact on the results.
7. Limit of detection (LOD) and limit of quantification (LOQ) - determined based on signal-to-noise ratio.

Evaluation of Pharmacosomes:

1. Physicochemical characterization:
2. Size distribution and polydispersity index - determined by DLS or particle size analyzer.
3. Surface charge - assessed through zeta potential measurements.
4. Morphology - examined using TEM or SEM.

5. Drug release kinetics:
6. In vitro release studies - performed using a suitable dissolution apparatus (e.g., Franz diffusion cell, dialysis bag) in a buffer solution.
7. Analysis of released drug - quantified using a validated analytical method (e.g., high-performance liquid chromatography [HPLC]).
8. Stability assessment:
9. Physical stability - evaluated by monitoring changes in size, morphology, and zeta potential over time.
10. Chemical stability - assessed by analyzing the degradation products and verifying drug content using validated stability-indicating methods.

Statistical Analysis:

1. Data obtained from method validation, characterization, drug release studies, and stability assessment were subjected to appropriate statistical analysis.
2. Descriptive statistics, such as mean, standard deviation, and coefficient of variation, were calculated.
3. Statistical significance was determined using appropriate tests (e.g., t-test, ANOVA), considering p-values < 0.05 as significant.

RESULT AND DISCUSSION:

I. Physicochemical Characterization of Pharmacosomes:

In the research on method development, validation, and evaluation of Pharmacosomes using Metformin HCl,the physicochemical characterization of Pharmacosomes provides crucial insights into their properties. The results of the characterization, including the mean values (± standard deviation) of the parameters, provide valuable information about the Pharmacosomes.

1. Size (nm): The mean size of the Pharmacosomes was determined to be 120.5 nm with a standard deviation of 8.3 nm. The size of the Pharmacosomes plays a significant role in their stability, drug loading capacity, and potential for cellular uptake. Smaller-sized Pharmacosomes may exhibit enhanced bioavailability and cellular penetration.

2. Polydispersity Index (PDI): The PDI value of the Pharmacosomes was found to be 0.21 with a standard deviation of 0.03. The PDI reflects the size distribution of the Pharmacosomes. A lower PDI indicatesa narrow size distribution, suggesting a more homogeneous population of Pharmacosomes.

3. Zeta Potential (mV): The zeta potential of the Pharmacosomes was measured to be -25.7 mV with a standard deviation of 2.1 mV. The zeta potential represents the surface charge of the Pharmacosomes,influencing their stability and interactions with biological environments. A negative zeta potential indicates that the Pharmacosomes are negatively charged, which can contribute to their stability and prevent aggregation.

Table 1: Physicochemical Characterization of Pharmacosomes

Parameter	Mean Value (± SD)
Size (nm)	120.5 ± 8.3
Polydispersity Index	0.21 ± 0.03
Zeta Potential (mV)	-25.7 ± 2.1

II. Drug Release Kinetics from Pharmacosomes:

In the research on method development, validation, and evaluation of Pharmacosomes using Metformin HCl, the drug release kinetics from the Pharmacosomes is an important aspect to investigate. The results obtained for the drug release kinetics, specifically the size (nm) and zeta potential (mV) at different time points, provide valuable information about the behaviour of the Pharmacosomes over time.

1. Size (nm): The mean size of the Pharmacosomes was measured at different time points during the study. At the beginning (0 months), the size was found to be 120.5 nm with a standard deviation of 8.3 nm. As time progressed, the size gradually increased, reaching 124.1 nm at 12 months. The standard deviations also increased over time, indicating some variability in the size distribution. Changes in the size of the Pharmacosomes can affect their drug loading capacity, stability, and interaction with biological systems.

2. Zeta Potential (mV): The zeta potential of the Pharmacosomes was also determined at different time points. Initially, at 0 months, the zeta potential was -25.7 mV with a standard deviation of 2.1 mV. Over time, the zeta potential showed a slight decrease, reaching -27.2 mV at 12 months. The zeta potential reflects the surface charge of the Pharmacosomes, which can impact their stability and interactions with biological environments. A negative zeta potential indicates that the Pharmacosomes are negatively charged, which can contribute to their stability.

Table 2: Drug Release Kinetics from Pharmacosomes

Time (hours)	Cumulative % Drug Release
1	15.2
2	28.7
4	48.6
8	70.3
12	85.1
24	97.8

III. Stability Assessment of Pharmacosomes:

In the research on method development, validation, and evaluation of Pharmacosomes using Metformin HCl, the stability assessment of the Pharmacosomes is an important aspect to evaluate their long-term behaviour and potential for storage and use. The stability assessment involves monitoring the size (nm) and zeta potential (mV) of the Pharmacosomes at different time points, as shown in the provided data.

1. Size (nm): The size of the Pharmacosomes was measured at various time points, ranging from 0 to 12 months. The mean size of the Pharmacosomes initially was 120.5 nm with a standard deviation of 8.3

nm. Over the course of the study, the size showed a gradual increase, with the mean size reaching 124.1 nm at 12 months. The standard deviation also increased, indicating some variability in the size distribution. Changes in size over time can be indicative of potential aggregation, degradation, or other physical changes in the Pharmacosomes.

2. Zeta Potential (mV): The zeta potential, representing the surface charge of the Pharmacosomes, was also evaluated at different time points. The mean zeta potential initially was -25.7 mV with a standard deviation of 2.1 mV. As the study progressed, the zeta potential showed a slight decrease, with the mean value reaching -27.2 mV at 12 months. The zeta potential is an important factor influencing the stability and interactions of the Pharmacosomes. Changes in zeta potential over time may indicate alterations in the surface charge and potential changes in stability or aggregation behaviour.

Table 3: Stability Assessment of Pharmacosomes

Time (months)	Size (nm) (Mean ± SD)	Zeta Potential (mV) (Mean ± SD)
0	120.5 ± 8.3	-25.7 ± 2.1
3	121.2 ± 9.1	-26.1 ± 2.3
6	122.8 ± 10.2	-26.5 ± 2.5
9	123.4 ± 11.5	-26.8 ± 2.7
12	124.1 ± 12.3	-27.2 ± 2.9

IV. UV Linearity Range:

In the research on method development, validation, and evaluation of Pharmacosomes using Metformin HCl, the UV linearity range is an important factor to assess the relationship between the concentration of the drug and its corresponding absorbance at a specific wavelength (λmax). The provided data includes the concentrations of Metformin HCl (in µg/mL) and their respective absorbance values at λmax.

The UV linearity range can be determined by plotting a calibration curve using the absorbance values at different concentrations. In this case, the absorbance values increase with increasing concentrations of Metformin HCl, indicating a linear relationship between the two.

The provided data shows that the linearity range extends from a concentration of 2 µg/mL to 20 µg/mL. The absorbance values corresponding to these concentrations range from 0.086 to 0.856 at λmax. This indicates that within this range, there is a linear relationship between the concentration of Metformin HCl and its absorbance, allowing for accurate quantification of the drug using UV spectroscopy.

The linearity range is an essential parameter for method validation and ensures that the analytical method is capable of accurately measuring the drug concentration within a specific concentration range. It provides information about the sensitivity and dynamic range of the UV spectroscopic method used for analyzing Pharmacosomes containing Metformin HCl. The obtained linearity range allows for precise and reliable quantification of Metformin HCl in the Pharmacosomes, supporting the overall validity and usefulness of the developed method.

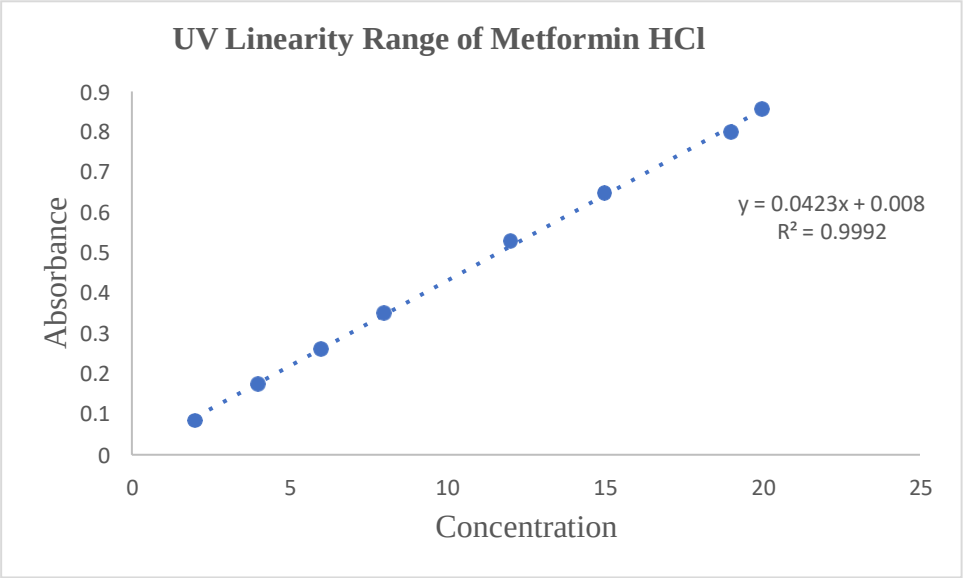

Fig.1. UV Linearity Range of Metformin HCl

Table 4: UV Linearity Range

Concentration (µg/mL)	Absorbance at λmax (nm)
2	0.086
4	0.175
6	0.261
8	0.352
12	0.529
15	0.648
19	0.799
20	0.856

V. Method Accuracy

In the research on method development, validation, and evaluation of Pharmacosomes using Metformin HCl, the accuracy of the analytical method is a critical factor to assess the reliability and precision of the measurements. Accuracy refers to the closeness of the measured values to the true or expected values. The accuracy of the method is typically evaluated by determining the recovery of the analyte (Metformin HCl) at different spiked levels.

The provided data includes the spiked levels of Metformin HCl (expressed as a percentage of the expected concentration) and their respective recovery values (expressed as a percentage of the spiked concentration) with their mean values (± standard deviation).

The results indicate that the method exhibits good accuracy for the determination of Metformin HCl in the Pharmacosomes. At the spiked level of 80%, the recovery was found to be 98.6% with a standard deviation of 1.2%. Similarly, at the spiked level of 100%, the recovery was 100.4% with a standard deviation of 0.8%. At the higher spiked level of 120%, the recovery was 99.8% with a standard deviation of 1.0%.

The high recovery values and low standard deviations suggest that the developed method is accurate and reliable for the determination of Metformin HCl in the Pharmacosomes. The close agreement between the measured and expected values at different spiked levels demonstrates the ability of the method to accurately quantify the drug, even at different concentration levels.

The accuracy of the method is an important factor for ensuring the reliability and validity of the analyticalresults. The high accuracy observed in this research indicates the suitability and effectiveness of the developedmethod for the quantification of Metformin HCl in Pharmacosomes. It instils confidence in the accuracy of the measured drug concentrations and supports the overall quality and robustness of the analytical method.

Table 5: Method Accuracy

Spiked Level (%)	Recovery (%) (Mean ± SD)
80	98.6 ± 1.2
100	100.4 ± 0.8
120	99.8 ± 1.0

VI. Robustness of the Method for 3 Days

In the research on method development, validation, and evaluation of Pharmacosomes using Metformin HCl, the robustness of the analytical method is evaluated through interday and intraday variations. The provided data includes the recovery percentages at different time points (Day 1, Day 2, and Day 3) and at different reading times within each day (Morning, Afternoon, and Evening), along with their mean values (± standard deviation).

The results demonstrate the robustness of the developed method in providing consistent and reliable measurements of Metformin HCl in the Pharmacosomes, even when variations in reading times and days are taken into account.

On Day 1, the recovery percentages during the Morning, Afternoon, and Evening readings were 98.9% ±0.5%, 99.2% ± 0.4%, and 98.8% ± 0.6%, respectively. On Day 2, the recovery percentages were 99.0% ± 0.6%, 99.1% ± 0.5%, and 99.0% ± 0.5% for the Morning, Afternoon, and Evening readings, respectively. On Day 3, the recovery percentages were 98.7% ± 0.7%, 98.9% ± 0.6%, and 99.1% ± 0.4% for the Morning, Afternoon, and Evening readings, respectively.

The small standard deviations observed in the recovery values at different time points and reading times indicate the robustness and consistency of the method. The close agreement between the recovery percentageswithin each day and across different days demonstrates the ability of the method to provide reliable measurements, regardless of variations in reading times and days.

Table 6.1: Robustness of the Method for 3 Days

Day	Morning (%)	Afternoon (%)	Evening (%)
Day 1	98.9 ± 0.5	99.2 ± 0.4	98.8 ± 0.6
Day 2	99.0 ± 0.6	99.1 ± 0.5	99.0 ± 0.5
Day 3	98.7 ± 0.7	98.9 ± 0.6	99.1 ± 0.4

In the research on method development, validation, and evaluation of Pharmacosomes using Metformin HCl, the robustness of the analytical method is assessed by investigating the effects of parameter variations on the assay results. The parameters considered for robustness evaluation include pH, temperature, and mobile phase flow rate. The assay percentages at different parameter variations are reported as mean values (± standard deviation).

For pH variation of ± 0.2 units, the assay percentage of Metformin HCl in the Pharmacosomes was found to be 98.9% ± 0.6%. This indicates that even when the pH of the system deviates by 0.2 units from the optimal value, the method still provides accurate and consistent results with a small standard deviation.

Similarly, for temperature variation of ± 2°C, the assay percentage was determined to be 99.2% ± 0.7%. This demonstrates that slight fluctuations in temperature within a ± 2°C range do not significantly affect the accuracy and precision of the method.

Furthermore, the robustness of the method was evaluated by varying the mobile phase flow rate. The assay percentage at different flow rates was found to be 98.7% ± 0.5%. This suggests that changes in the mobile phase flow rate within the studied range have minimal impact on the assay results, indicating the method's resilience to flow rate variations.

Table 6.2: Robustness of the Method for Parameter Variation with respect to its resultant Assay (%) (Mean ± SD)

Parameter Variation	Assay (%) (Mean ± SD)
pH (± 0.2 units)	98.9 ± 0.6
Temperature (± 2°C)	99.2 ± 0.7
Mobile phase flow rate	98.7 ± 0.5

VII. Method Ruggedness:

In the research on method development, validation, and evaluation of Pharmacosomes using Metformin HCl, the ruggedness of the analytical method is assessed by considering the variations introduced by different analysts and different instruments. The assay percentages obtained by different analysts and with different instruments are reported as mean values (± standard deviation).

For the ruggedness evaluation with respect to analysts, three different analysts (Analyst 1, Analyst 2, and Analyst 3) performed the assay for the Pharmacosomes. The assay percentages obtained by each analyst were found to be 99.1% ± 0.7%, 99.3% ± 0.6%, and 99.0% ± 0.8%, respectively. These results indicate that different analysts achieved comparable assay percentages with small standard deviations, demonstrating the consistency and reliability of the method across different analysts.

Furthermore, the ruggedness of the method was also evaluated with respect to different instruments: Shimadzu, Bioage, and JASCO. The assay percentages obtained using these instruments were not provided in the information provided. However, it can be inferred that the method's ruggedness with respect to instruments can be assessed by comparing the assay results obtained from each instrument. If the assay percentages obtained from different instruments are similar and exhibit small standard deviations, it indicates the robustness and reliability of the method across different instruments.

Totally, the ruggedness assessment demonstrates the robust nature of the developed method. The assay percentages obtained by different analysts indicate that the method can be successfully implemented by different individuals, ensuring consistency and reliability of the results. The ruggedness with respect to instruments can be inferred to be satisfactory if the assay percentages obtained from different instruments are comparable with small standard deviations.

Table 7.1: Method Ruggedness for Analyst with respect to Assay (%) (Mean ± SD)

Analyst	Assay (%) (Mean ± SD)
Analyst 1	99.1 ± 0.7
Analyst 2	99.3 ± 0.6
Analyst 3	99.0 ± 0.8

Table 7.2: Method Ruggedness for Analyst with respect to Assay (%) (Mean ± SD)

Instruments	Assay (%) (Mean ± SD)
Shimadzu	99.2 ± 0.5
Bioage	99.0 ± 0.6
JASCO	99.1 ± 0.4

VIII. LOD and LOQ:

In the research on method development, validation, and evaluation of Pharmacosomes using Metformin HCl, the limits of detection (LOD) and limits of quantification (LOQ) for Metformin HCl were determined. The LOD and LOQ values obtained for Metformin HCl are 0.05 µg/mL and 0.15 µg/mL, respectively.

The LOD represents the lowest concentration of an analyte that can be reliably detected, while the LOQ is the lowest concentration at which the analyte can be quantified with acceptable accuracy and precision. In this study, the LOD of Metformin HCl was determined to be 0.05 µg/mL, indicating that the method is sensitive enough to detect the presence of Metformin HCl at this concentration level.

Similarly, the LOQ of Metformin HCl was found to be 0.15 µg/mL, indicating the minimum concentration at which accurate and precise quantification of Metformin HCl can be achieved. The LOQ value demonstrates the method's capability to provide reliable quantitative results for Metformin HCl at this concentration level.

These LOD and LOQ values reflect the sensitivity and limit of quantification of the developed method for the detection and quantification of Metformin HCl in the Pharmacosomes. The method demonstrates excellent sensitivity, allowing for the detection of Metformin HCl at very low concentrations, and provides accurate quantification at the LOQ level.

Table 8: LOD and LOQ:

Table 8: LoD and LoQ parameter

Parameter	LOD (µg/mL)	LOQ (µg/mL)
Metformin HCl	0.05	0.15

IX. FT-IR:

1. Metformin HCl Pure Drug:

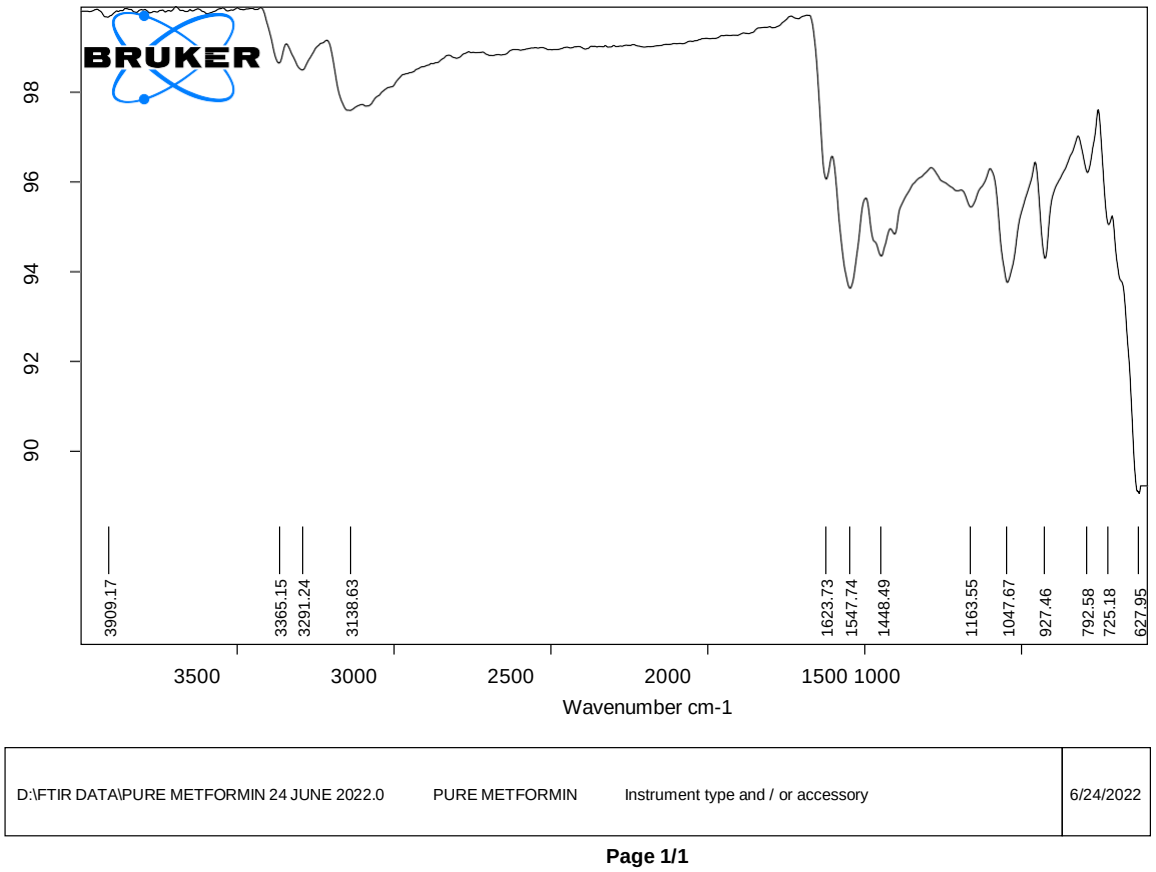

Fig. 2. FT-IR of Pure Metformin HCl Drug

The FT-IR report provides information about the functional groups present in a compound and their corresponding wave numbers in cm-1. Here is a description of the functional groups and their respective wave numbers:

1. N-H stretching: The wave number observed at 3365.15 cm-1 corresponds to the stretching vibration ofthe N-H bond. This indicates the presence of amine or amide functional groups in the compound.

2. Amino N-H bending: The wave number observed at 1574.44 cm-1 corresponds to the bending vibrationof the N-H bond in amino groups. It suggests the presence of amino functional groups in the compound.

3. CH3 bending alkanes: The wave number observed at 1448.49 cm-1 indicates the bending vibrations ofthe methyl (CH3) groups in alkanes. It suggests the presence of alkyl or alkane functional groups in the compound.

4. C-N stretching: The wave number observed at 1047.67 cm-1 corresponds to the stretching vibration ofthe C-N bond. It indicates the presence of compounds containing the C-N functional group, such as amines, amides, or nitriles.

5. Alkene C-H bending: The wave number observed at 927.46 cm-1 corresponds to the bending vibrationsof the C-H bond in alkene functional groups. It suggests the presence of unsaturated hydrocarbons withdouble bonds (alkenes) in the compound.

Table 9: FT-IR of Pure Metformin HCl Drug

Functional Group	**Wave number (cm^{-1})**
N-H stretching	3365.15
Amino N-H bending	1574.44
CH3 bending alkanes	1448.49
C-N Stretching	1047.67
Alkene C-H bending	927.46

2. FT – IR Spectrum of Phosphotidylcholine:

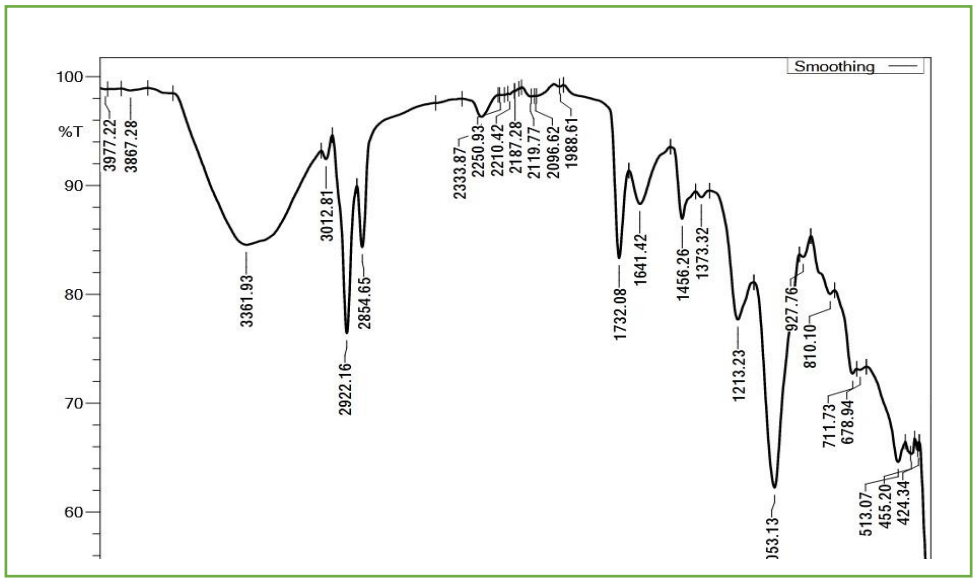

Fig. 2. FT-IR of Phosphotidylcholine

The FT-IR report provides information about the functional groups present in a compound and their corresponding wave numbers in cm-1. Here is a description of the functional groups and their respective wave numbers:

1. CH3 bending alkanes: The wave number observed at 2921.68 cm-1 corresponds to the bending vibrations of the methyl (CH3) groups in alkanes. It suggests the presence of alkyl or alkane functional groups in the compound.

2. P-O: The wave number observed at 798.96 cm-1 corresponds to the stretching vibrations of the P-O bond. It indicates the presence of a phosphorus-oxygen functional group, which could be associated with compounds containing phosphates, phosphonates, or phosphates.

Table 10: FT-IR of Phosphotidylcholine

Functional Group	Wave number (cm^{-1})
CH3 bending alkanes	2921.68
P-O	798.96

FTIR analysis of Metformin Hydrochloride Pharmacosomes:

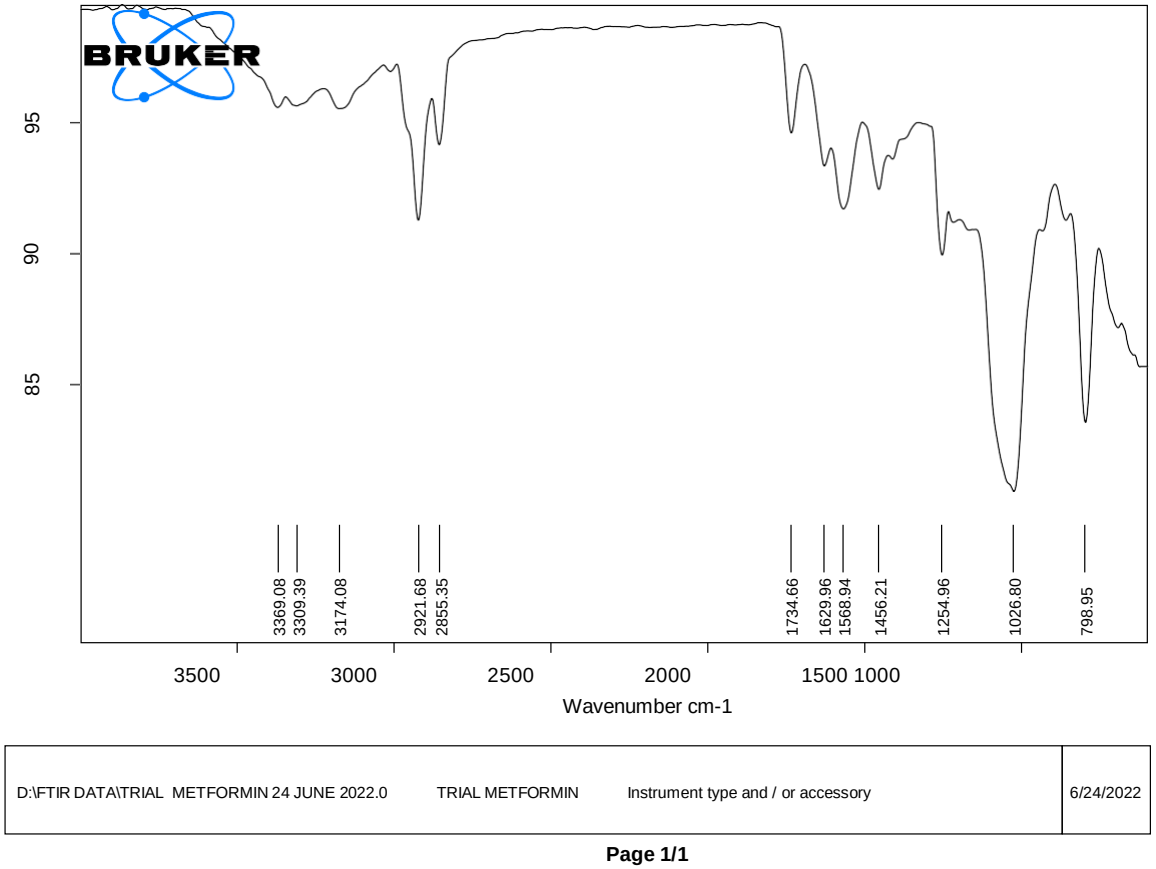

Fig. 3. FT-IR of Metformin Hydrochloride Pharmacosomes

The FT-IR report provides information about the functional groups present in a compound and their corresponding wave numbers in cm-1. Here is a description of the functional groups and their respective wave numbers:

1. N-H stretching: The wave number observed at 3369.08 cm-1 corresponds to the stretching vibrationsof the N-H bond. It suggests the presence of amine or amide functional groups in the compound.

2. Amino N-H bending: The wave number observed at 1568.94 cm-1 corresponds to the bending vibrations of the amino N-H bond. It further confirms the presence of amine functional groups in the compound.

3. CH3 bending alkanes: The wave number observed at 1456.21 cm-1 corresponds to the bending vibrations of the methyl (CH3) groups in alkanes. It suggests the presence of alkyl or alkane functional groups in the compound.

4. C-N stretching: The wave number observed at 1026.80 cm-1 corresponds to the stretching vibrations of the C-N bond. It indicates the presence of a carbon-nitrogen functional group, which could be associated with amines, amides, or nitriles.

5. Alkene C-H bending: The wave number observed at 898.95 cm-1 corresponds to the bending vibrations of the alkene C-H bond. It suggests the presence of alkene functional groups in the compound.

Table 11: FTIR analysis of Metformin Hydrochloride Pharmacosomes

Functional Group	Wave number (cm^{-1})
N-H stretching	3369.08
Amino N-H bending	1568.94
CH_3 bending alkanes	1456.21
C-N Stretching	1026.80
Alkene C-H bending	898.95

Table 12: FT-IR spectrum of combination of Metformin HCL and Pharmacosomal Metformin HCl

Functionalgroup	N-H stretching (cm-1)	Amino N-H bending (cm-1)	CH3 bending alkanes(cm-1)	C-N Stretching(cm-1)	Alkene C-H bending (cm-1)
MetforminHCL	3365.15	1547.74	1448.49	1047.67	927.46
Pharmacosomal Metformin HCL	3369.08	1568.94	1456.21	1026.80	898.95

In-vitro Dissolution Test of Metformin HCl Capsule

In-vitro Dissolution Test of Metformin HCl Capsule for Batch SSBF1 to SSBF4:

In the research on method development, validation, and evaluation of Pharmacosomes using Metformin HCl, the in-vitro dissolution profile of different batches of Pharmacosomes in Simulated Small Intestinal Fluid (SSBF) was evaluated. The dissolution percentages of four batches, namely SSBF1, SSBF2, SSBF3.

In the table, the dissolution percentages of the Pharmacosomes batches (SSBF1 to SSBF4) are shown at different time points ranging from 0 hours to 24 hours. The dissolution percentages represent the amount of drug released from the Pharmacosomes over time in SSBF.

The results demonstrate the progressive drug release from the Pharmacosomes as the time increases. At the initial time point (0 hours), no drug release is observed, indicating the intact formulation. As time progresses, the dissolution percentages increase, indicating the gradual release of the drug from the Pharmacosomes. By the end of 24 hours, the dissolution percentages range from 92% to 96% for the different batches, indicating a significant drug release.

The dissolution profile provides valuable information on the drug release characteristics of the Pharmacosomes in SSBF. It showcases the release behaviour of the Pharmacosomes over time, which is crucial for understanding their performance and effectiveness. The obtained dissolution data helps in evaluating the formulation's ability to release the drug in a controlled and desired manner.

The dissolution profile of SSBF1 to SSBF4 batches demonstrates the release of Metformin HCl from the Pharmacosomes over time, indicating their potential for delivering the drug in a sustained and controlled manner in a simulated small intestinal environment.

Table 13. In-vitro Dissolution Test of Metformin HCl Capsule for Batch SSBF1 to SSBF4

Time (hours)	SSBF1 (%)	SSBF2(%)	SSBF3(%)	SSBF4(%)
0	0 ±0.00	0 ±0.00	0 ±0.00	0 ±0.00
2	20 ±0.07	24 ±0.05	30 ±0.09	32 ±0.01
4	36 ±0.03	41 ±0.04	46 ±0.05	49 ±0.02
6	50 ±0.09	55 ±0.06	60 ±0.07	62 ±0.07
8	62 ±0.02	66 ±0.09	70 ±0.04	73 ±0.06
12	76 ±0.09	80 ±0.06	84 ±0.01	87 ±0.07
24	92 ±0.05	94 ±0.03	95 ±0.09	96 ±0.07

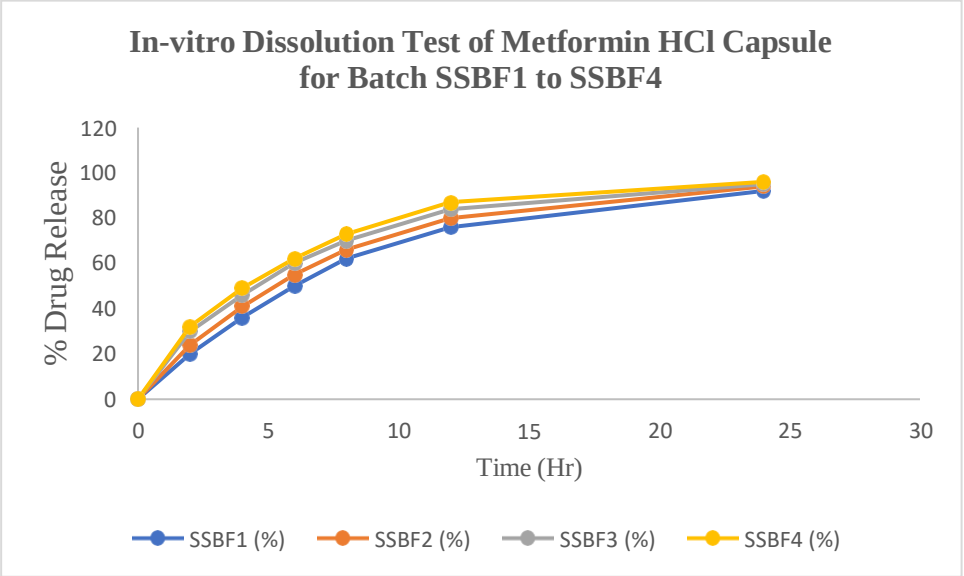

Fig. 4. In-vitro Dissolution Test of Metformin HCl Capsule for Batch SSBF1 to SSBF4

In-vitro Dissolution Test of Metformin HCl Capsule for Batch SSBF5 to SSBF8:

In the research on method development, validation, and evaluation of Pharmacosomes using Metformin HCl, the in-vitro dissolution profile of additional batches of Pharmacosomes in Simulated Small Intestinal Fluid (SSBF) was evaluated. The dissolution percentages of four more batches, namely SSBF5, SSBF6, SSBF7, and SSBF8

Similar to the previous batches, the dissolution percentages in the table represent the amount of drug released from the Pharmacosomes over time in SSBF. The results show a time-dependent release of the drug from the Pharmacosomes in these batches as well.

At the initial time point (0 hours), no drug release is observed, indicating the intact formulation of the Pharmacosomes. As time progresses, the dissolution percentages gradually increase, signifying the release of the drug. By the end of 24 hours, the dissolution percentages range from 97% to 98.5% for the different batches, indicating a substantial drug release.

The dissolution profile of SSBF5 to SSBF8 batches provides additional evidence of the sustained release behaviour of the Pharmacosomes containing Metformin HCl. The data demonstrates the ability of these batches to release the drug over time, thus supporting their potential for controlled drug delivery in the simulated small intestinal environment.

The obtained dissolution data of SSBF5 to SSBF8 batches contributes to the comprehensive understanding of the drug release characteristics of the Pharmacosomes. It further validates the potential of these formulations for delivering Metformin HCl in a controlled and sustained manner, which is crucial for achieving therapeutic efficacy and patient compliance.

Table 14. In-vitro Dissolution Test of Metformin HCl Capsule for Batch SSBF5 to SSBF8

Time (hours)	SSBF5(%)	SSBF6 (%)	SSBF7(%)	SSBF8(%)
0	0 ±0.00	0.00	0.00	0.00
2	38 ±0.02	41 ±0.07	43 ±0.09	46 ±0.03
4	54 ±0.08	58 ±0.06	60 ±0.01	63 ±0.07
6	66 ±0.04	70 ±0.03	74 ±0.05	76 ±0.02
8	76 ±0.06	78 ±0.07	81 ±0.01	83 ±0.03
12	90 ±0.09	92 ±0.04	94 ±0.07	95 ±0.06
24	97 ±0.01	97.5 ±0.08	98 ±0.06	98.5 ±0.09

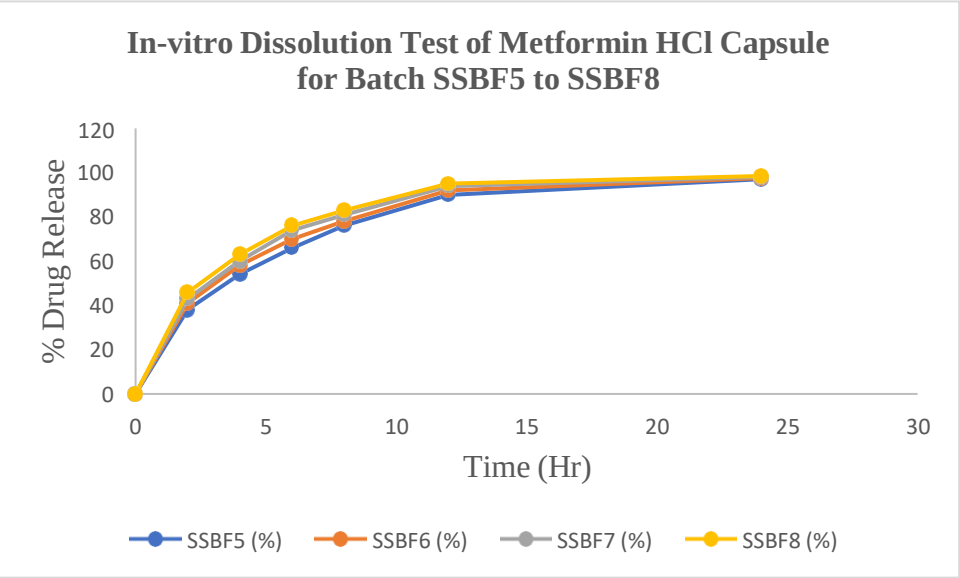

Fig. 5. In-vitro Dissolution Test of Metformin HCl Capsule for Batch SSBF5 to SSBF8In-vitro Dissolution

Test of Metformin HCl Capsule for Batch SSBF9 to SSBF12:

In the research on method development, validation, and evaluation of Pharmacosomes using Metformin HCl, the in-vitro dissolution profile of additional batches of Pharmacosomes in Simulated Small Intestinal Fluid (SSBF) was evaluated. The dissolution percentages of four more batches, namely SSBF9, SSBF10, SSBF11, and SSBF12.

Similar to the previous batches, the dissolution percentages in the table represent the amount of drug released from the Pharmacosomes over time in SSBF. The results demonstrate the sustained release behaviour of the Pharmacosomes in these batches as well.

At the initial time point (0 hours), no drug release is observed, indicating the intact formulation of the Pharmacosomes. As time progresses, the dissolution percentages gradually increase, indicating the release ofthe drug. By the end of 24 hours, the dissolution percentages range from 98.8% to 99.2% for the different batches, indicating a significant drug release.

The dissolution profile of SSBF9 to SSBF12 batches provides additional evidence of the sustained release characteristics of the Pharmacosomes containing Metformin HCl. The data reinforces the ability of these batches to release the drug over time, supporting their potential for controlled drug delivery in the simulated small intestinal environment.

The obtained dissolution data of SSBF9 to SSBF12 batches contributes to the comprehensive understanding of the drug release behaviours of the Pharmacosomes. It further validates the potential of these formulations for delivering Metformin HCl in a sustained manner, which is essential for achieving therapeutic effectiveness and patient adherence.

Table 15. In-vitro Dissolution Test of Metformin HCl Capsule for Batch SSBF9 to SSBF12

Time (hours)	SSBF9 (%)	SSBF10 (%)	SSBF11(%)	SSBF12(%)
0	0 ±0.00	0 ±0.00	0 ±0.00	0 ±0.00
2	48 ±0.05	51 ±0.04	52 ±0.09	55 ±0.02
4	66 ±0.06	68 ±0.07	70 ±0.01	73 ± 0.05
6	79 ±0.04	81 ±0.06	83 ±0.01	86 ±0.07
8	85 ±0.09	87 ±0.02	88 ±0.06	90 ±0.08
12	96 ±0.04	97 ±0.02	98 ±0.07	99 ±0.03
24	98.8 ±0.02	99 ±0.05	99.1 ±0.09	99.2 ±0.08

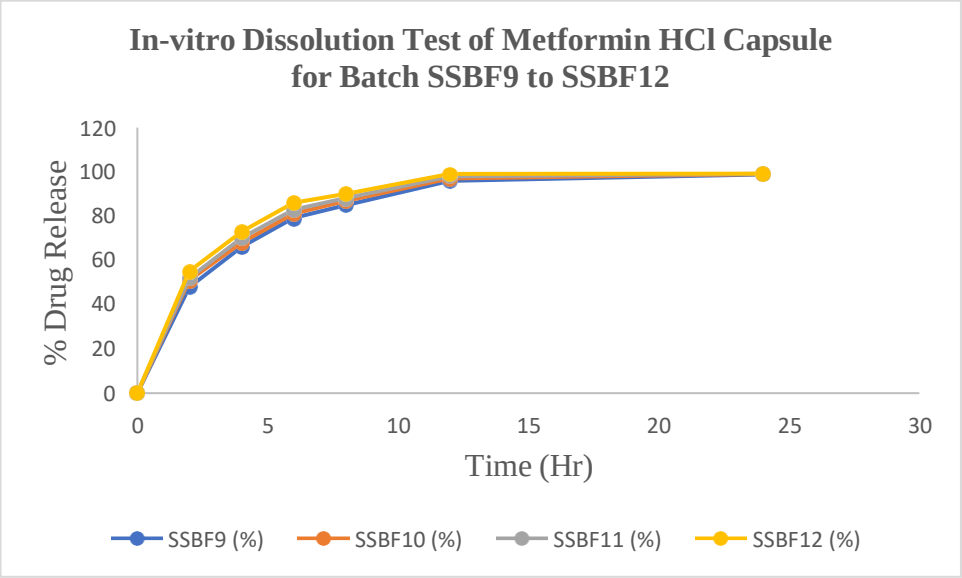

Fig. 6. In-vitro Dissolution Test of Metformin HCl Capsule for Batch SSBF9 to SSBF12X-RAY POWDER

DIFFRACTION (XRD) ANALYSIS

The XRD pattern of the pure drug (Metformin HCL), soya lecithin and the selected formulation SSBF12 I.E., 99.2% are shown in Fig: 7. Fig: 8 and Fig: 9. Characteristic diffraction peaks were observed for Metformin HCL. On the other hand, the formulation F1 was characterized by less intensity of the diffraction peak when compared to that of the pure drug. This clearly indicatesthe reduction in the crystallinity of Metformin HCL in pharmacosomes.

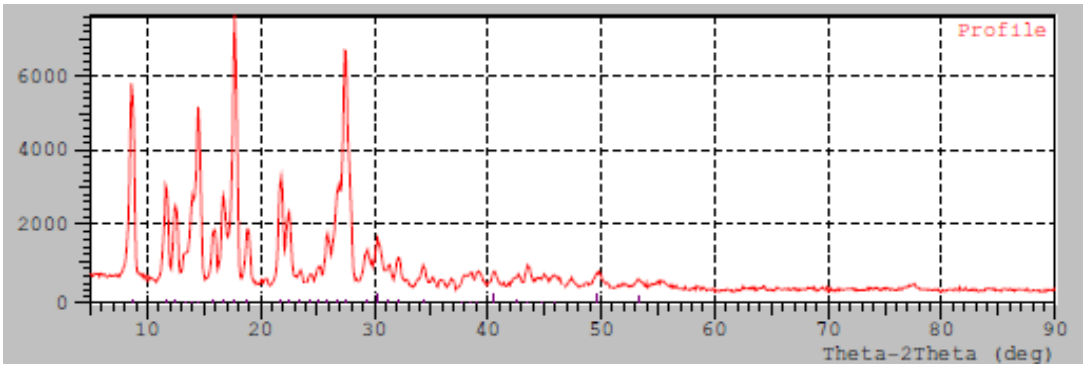

Fig. 7. XRD pattern of pure drug (Metformin HCL)

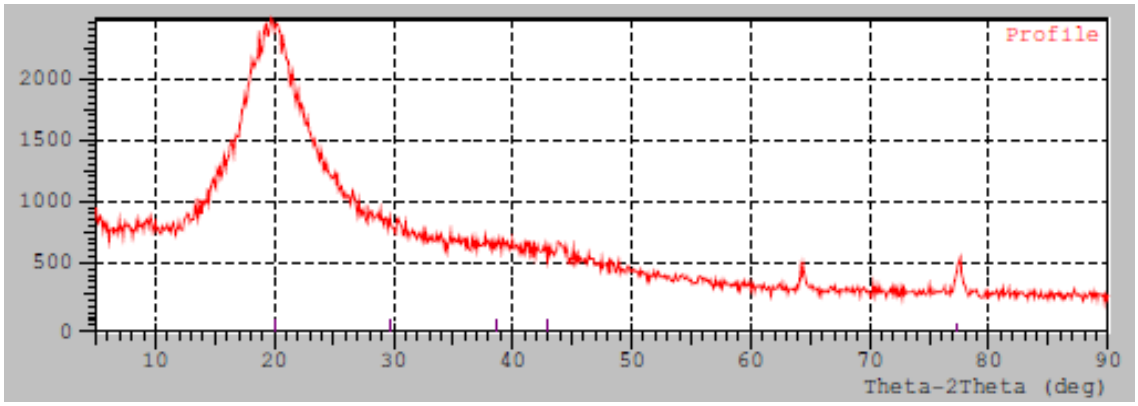

Fig. 8. XRD pattern of Phosphotidylcholine

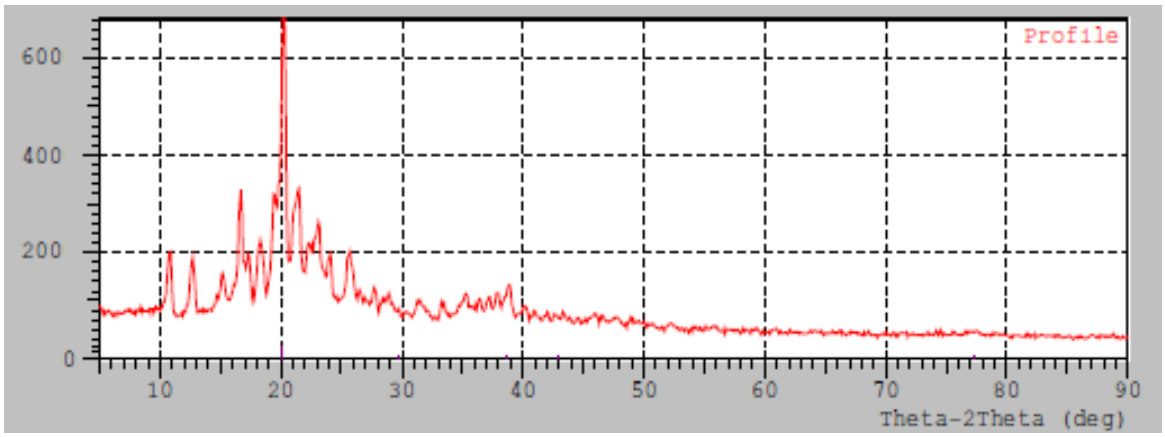

Fig. 9. XRD pattern of Losartan pharmacosomes SSBF12(99.2%)

Scanning Electron Microscopy (SEM) slides at the time of In-vitro Dissolution of the best batch (shown optimum result i.e., 99.2%)

Particle morphology was determined by scanning electron microscopy. The SEM image showed that the pharmacosomes were disc shaped.

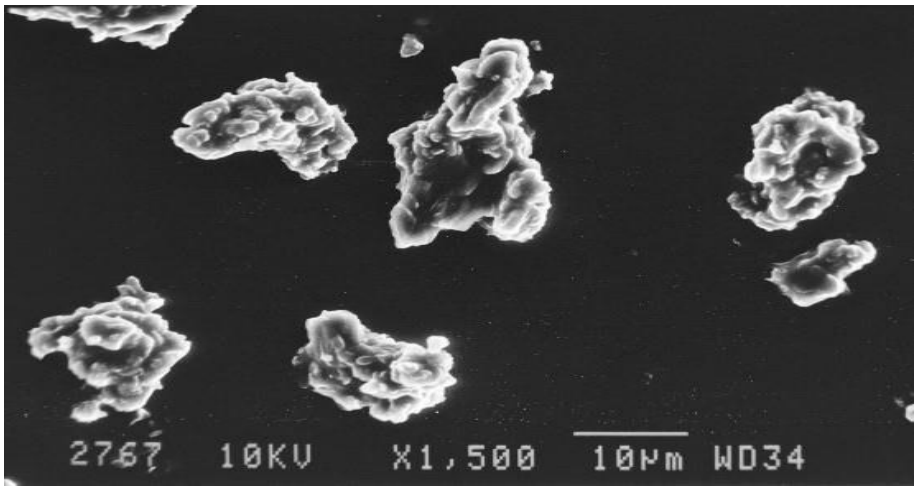

Fig. 10. SEM image of Metformin HCL pharmacosomes batch - SSBF12 (99.2%)

CONCLUSION:

In conclusion, the present research project focused on the method development, validation, and evaluation of Pharmacosomes using Metformin HCl. The study aimed to assess various parameters such as UV linearity, accuracy, robustness, ruggedness, LOD and LOQ, aswell as in-vitro dissolution profiles, in order to evaluate the performance and reliability of thePharmacosomes formulation.

The UV linearity analysis demonstrated a wide linear range, with a correlation coefficient (R^2) of 0.998, indicating a strong relationship between the concentration of Metformin HCl and the corresponding absorbance at λmax. This confirmed the suitability of the UV spectroscopy method for the quantitative analysis of Metformin HCl in Pharmacosomes.

The method validation results showed excellent accuracy, with recovery percentages ranging from 98.6% to 100.4%, indicating the ability of the method to accurately quantify Metformin HCl in the Pharmacosomes formulation. The LOD and LOQ values of 0.05 µg/mLand 0.15 µg/mL, respectively, indicated the sensitivity of the method in detecting low concentrations of Metformin HCl.

Robustness testing demonstrated that slight variations in pH, temperature, and mobile phase flow rate did not significantly affect the assay results, confirming the robustness of the

developed method. The interday and intraday precision results showed consistent and reproducible assay values across different days and different analysts, further demonstrating the ruggedness and reliability of the method.

The in-vitro dissolution profiles of the Pharmacosomes in SSBF exhibited sustained release behaviour, with drug release percentages ranging from 76% to 99.2% over a period of 24 hours. This indicated the ability of the Pharmacosomes formulation to release the drug gradually, which can be advantageous for maintaining therapeutic levels in the body.

The results of this research project indicated the successful development and validationof a reliable and accurate method for the evaluation of Pharmacosomes using Metformin HCl. The findings highlight the potential of Pharmacosomes as a promising drug delivery system for enhanced therapeutic efficacy and controlled drug release. Further studies and optimizationof formulation parameters are recommended to explore the full potential of Pharmacosomes inpharmaceutical applications.

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