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Development And Optimization Of Picroside Ii Capsules For The Treatment Of Liver Disease Using Quality By Design Approach.

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ABSTRACT

The proposed research work explores the systematic development and optimization of Picroside II capsules employing the Quality by Design (QbD) approach. Picroside II, a bioactive compound derived from *Picrorhiza kurroa* rhizomes, has exhibited potential therapeutic properties, making it an attractive candidate for formulation in pharmaceutical dosage forms. The study integrates the principles of QbD, a contemporary pharmaceutical quality management paradigm, to enhance the robustness and efficiency of the formulation process. This study is aimed at formulating solid oral dosage forms of picroside II for the treatment of liver disease. The flow property of the picroside II formulation was determined. The formulated capsules passed pharmacopeia tests, including evaluations of weight uniformity, disintegration, drug content, and dissolution. The formulation was excellent flow characteristics. The prepared capsules exhibited excellent in vitro release of the extract, with more than 90% released within 30 minutes. Additionally, they effectively fulfilled the requirements for uniformity of weight, disintegration, and drug content testing. The results obtained from these evaluations are examined to assess the efficacy of the QbD-driven formulation method in attaining the intended product quality.

The findings of this research contribute to the systematic understanding of the formulation process for Picroside II capsules, emphasizing the importance of QbD principles in ensuring the reproducibility and reliability of pharmaceutical formulations. The application of QbD in this study provides a valuable template for the development of other herbal-based pharmaceuticals, fostering the advancement of evidence-based traditional medicine in the modern pharmaceutical landscape. Picroside II capsules have been successfully formulated and are available to use as conventional dosage forms for the treatment of liver diseases.

KEYWORDS: Picroside II, Weight variation, In-Vitro Drug Release, QbD approach

1. INTRODUCTION

Since ancient era, human beings are using natural resources as food as well as medicine. In case of natural medicines, there is a huge literature of plant material as significant source of medicines. In India, Ayurveda is one of important systems of medicine. In Ayurveda, number of plants is described for their great therapeutic potential. Recently, considering the side effects of Allopathic system of medicine, people are widely accepting the Ayurveda as their primary system of medicine [1-2]. Ayurveda system of medicine has its own plant collection methodology, preparation schemes and the dosage regimen. Although, said system of medicine offers promising therapeutic benefits, it does not possess standardization mechanisms. Due to change in climate, collection period and processing parameters, there are chances of change in magnitude of therapeutic benefits of plant-based medicines. [3].

The development of stable herbal formulations is particularly challenging due to the diverse chemical components present in medicinal plants. The entire herbal drug or preparation is considered an active drug substance, lacking standardized controls, which underscores the need for robust studies akin to allopathic drug discovery and development processes. Applying such rigorous methodologies to naturally occurring therapeutic agents holds the potential to transform them into consistent and effective drug moieties [4-5].

While performing extensive literature survey, it was observed that, around 400 million people all over the world are suffering from chronic hepatic disorders and Asia-Pacific region is the epicenter of this disorder. It is well reported that there is no direct remedy in allopathy for hepatotoxicity. All of the anti-hepatotoxic or hepatoprotective agents are of natural origin. In spite of therapeutic potential of such agents, the scientific data in terms of recent drug discovery and development process is

missing [6-7].

Picrorrhiza kurroa Royle (Scrophulariaceae), commonly known as Kutki is a well-known herb in ayurvedic medicine. Picrorrhiza kurroa is a low, hairy herb with a perennial woody rhizome. Traditionally P. kurroa has been used to treat disorders of the liver and upper respiratory tract. Its pharmacological studies have revealed hepatoprotective, anti-inflammatory, immunostimulatory and free-radical scavenging activity. The hepatoprotective value of the Picrorrhiza kurroa has been attributed to the presence of picroside II. PK-II exhibits a wide range of pharmacological activities such as hepatoprotective, anti-inflammatory, antioxidant activity, anti-diabetic, anti-allergic Activity and anticancer potential. PK II used to support liver health and protect the liver from damage caused by toxins, drugs, and various diseases [8-11].

The research is novel in its focus on naturally occurring hepatoprotective agents and their implications in drug development. By studying lesser-explored compounds, the study brings fresh perspectives to the field, potentially uncovering new therapeutic options for liver diseases.

2. MATERIAL AND METHOD

Chemicals and reagents

Picroside- II (Purity 99.2% by HPLC) in house isolated from *Picrorhiza kurroa rhizomes*. All the chemicals of analytical grade were used for the proposed study.

Integration of Quality by Design (QbD) Principles

The formulation approach was guided by the principles of Quality by Design (QbD), which underscores the significance of a systematic and scientific formulation process. Within this framework, a Box-Behnken design was employed as an experimental strategy to systematically assess the impact of various formulation and process variables on the final characteristics of the capsules [12-13].

Statistical Software

Design-Expert, a specialized statistical software package, was utilized for the experimental design, analysis of variance (ANOVA), and graphical representation of the results. The software allowed for the identification of significant factors and their interactions, thereby aiding in the optimization of the formulation.

Experimental Design

A Box-Behnken design was employed to systematically investigate the effects of various formulation factors on the performance characteristics of the PK II capsules. The independent variables considered for the design were Microcrystalline Cellulose (MCC) concentration, Magnesium Stearate concentration, Sodium Starch Glycolate (SSG) concentration, and Sodium Lauryl Sulfate (SLS) concentration. These factors were varied at different levels according to the design matrix.

Experimental Design Variables and Their Levels

The selected variables and levels are depicted in table 1 and the different batches of experimental design and compositions are shown in table 2.

Table 1. Formulation and process variables & level

Independent variables	Unit	Coded Symbol	Levels		
			-1	0	+1
Concentration of Microcrystalline Cellulose	%	A	20%	45%	90%
Concentration of Magnesium Stearate	%	B	0.25%	2.50%	5.00%
Concentration of Sodium Starch Glycolate	%	C	2%	4%	8%
Concentration of Sodium Lauryl Sulfate	%	D	1%	1.50%	2.00%

Table 2. Experimental design for capsule manufacturing

Batch	Factor-1 MCC concentration (%)	Factor-2 Mag. Stearate concentration (%)	Factor-3 SSG concentration (%)	SLS concentration (%)
PK-1	90	0.25	5	1.5
PK -2	20	2.625	2	1.5
PK -3	55	5	8	1.5
PK -4	90	2.625	5	2
PK -5	55	2.625	2	1
PK -6	55	0.25	2	1.5
PK -7	90	5	5	1.5
PK -8	55	2.625	5	1.5
PK -9	55	5	5	1
PK -10	20	5	5	1.5
PK -11	55	2.625	5	1.5
PK -12	55	5	5	2
PK -13	55	2.625	5	1.5
PK -14	90	2.625	2	1.5
PK -15	55	2.625	2	2
PK -16	55	2.625	8	2
PK -17	55	2.625	8	1
PK -18	20	2.625	5	1
PK -19	55	0.25	5	2
PK -20	20	2.625	5	2
PK -21	90	2.625	8	1.5
PK -22	55	2.625	5	1.5
PK -23	20	2.625	8	1.5
PK -24	55	5	2	1.5
PK -25	55	2.625	5	1.5
PK -26	90	2.625	5	1
PK -27	55	0.25	5	1
PK -28	55	0.25	8	1.5
PK -29	20	0.25	5	1.5

Preparation of capsule formulation

Accurately measure the predetermined quantities of PK-II, MCC, Magnesium stearate, SSG and SLC (Table 2). Homogeneous blending of the excipient to attain a uniform mixture, ensuring an even distribution of each component within the formulation. Load the blended mixture into empty capsule using a manual capsule filling machine. Meticulously ensuring consistent filling to avoid variations between individual capsules. Seal the capsules to prevent any potential leakage, ensuring the integrity of the formulation and extending the shelf life of the product [14-15].

Quality Assessment of Formulated Capsules

Flow properties of picroside II formulation

Flow properties or rheological studies of the picroside II capsule formulation were characterized by measuring the angle of repose, Carr's compressibility index and Hausner's ratio. The angle of repose was by the fixed funnel method whereas; Carr's compressibility index and Hausner's ratio were calculated from the bulk and tapped density of the picroside II [16].

General Appearance Evaluation

The evaluation of the general appearance of the PK II capsules encompassed a comprehensive assessment of their external characteristics. This examination aimed to identify any potential defects, pinholes, irregularities, or variations in texture and appearance. The capsules were observed under suitable lighting conditions, employing the naked eye for visual analysis. This process allowed for the initial screening of the capsules' physical attributes, providing insights into their overall quality and visual integrity.

Weight Variation Analysis

Twenty capsules were randomly chosen from the batch, and their individual weights were determined. The average weight of the selected capsules was calculated, serving as the reference for comparison. Each individual capsule's weight was then compared to the average weight to identify any significant variations. This analysis provided insights into the consistency of the capsule filling process and the uniformity of the capsule weights.

Disintegration Test

The disintegration test evaluated the time required for the PK II capsules to disintegrate in a specific environment. One capsule was introduced into each tube of the disintegration apparatus. The assembled apparatus was then suspended in a beaker containing 60 ml of water maintained at 37°C. If any hard capsules floated on the water's surface, a disc was added to facilitate the test. The apparatus was operated for 30 minutes, after which it was removed from the liquid. The residue remaining on the screen of the apparatus was observed and recorded, providing information on the capsules' disintegration behavior.

Drug Content Analysis

For the determination of the drug content in the PK II capsules, a total of 20 capsules from each formulation were selected. The powder contents of the capsules were extracted and transferred to a 100 ml volumetric flask. The extracted powder was dissolved in distilled water using a sonicator for 10-15 minutes. The resulting solution was filtered, appropriate dilutions were prepared, and the absorbance was measured using High-Performance Liquid Chromatography (HPLC). This analysis allowed for the quantification of the actual drug content present in the capsules, ensuring their potency and adherence to specified standards.

Method for Drug-Excipient Compatibility Study by FTIR

A comprehensive drug-excipient compatibility study was meticulously conducted using Fourier Transform Infrared Spectroscopy (FTIR) to assess the potential chemical interactions between PK II (the active pharmaceutical ingredient) and various excipients, including Magnesium Stearate, Microcrystalline Cellulose (MCC), Sodium Lauryl Sulfate (SLS), and Sodium Starch Glycolate. This study was pivotal in determining the suitability of these excipients for the formulation of PK II capsules by evaluating whether any interactions could compromise the therapeutic integrity or quality of the final product. The FTIR analysis comprised several crucial steps. First, the individual FTIR spectra of PK II and each excipient were recorded to establish their characteristic absorption bands and peak positions. Subsequently, physical mixtures were prepared by combining PK II with each excipient in various proportions. FTIR spectra of these mixtures were then collected to capture any changes in peak intensities or shifts in characteristic bands, which could signify possible interactions [17-18].

Differential Scanning Calorimetry (DSC) of PK II Formulation

The evaluation of the PK II formulation's thermal behavior was conducted using Differential Scanning Calorimetry (DSC), a technique employed to investigate heat flow associated with phase transitions and thermal events in materials. A representative sample of the PK II formulation was accurately weighed and placed in an aluminum pan, while an empty pan served as the reference. The pans were hermetically sealed to prevent gas exchange. The analysis was performed using a DSC-60 Plus (Shimadzu) instrument, applying the following conditions: a temperature range of 10°C to 300°C, a heating rate of 10^0C/min, and a liquid nitrogen atmosphere [19].

FTIR Study of PK II Formulation

The Fourier Transform Infrared (FTIR) spectroscopy technique was employed to investigate the molecular composition and functional groups present in the PK II formulation. A representative sample of the formulation was prepared for analysis. The FTIR spectra were recorded using an IR Affinity-1S (Shimadzu) FTIR instrument operating in the 7800 to 350 cm^{-1} mode. The spectral range used for the analysis was 4000cm^{-1} to 400 cm^{-1}. The obtained FTIR spectra were compared to reference spectra of the individual excipients and PK II to identify and analyze the characteristic peaks corresponding to specific functional groups [20-21].

***In-Vitro* Dissolution Release**

The *in-vitro* dissolution release assessment was conducted utilizing a Dissolution Test Apparatus-2, following the guidelines outlined in USP-32. The test was carried out using the Basket Apparatus. The release media was placed into each of the six dissolution vessels, with each vessel containing 900mL of 0.1M HCl. The water jacket was heated to a temperature of 37 ± 2°C, which is intended to simulate the temperature of the human body. Then, a capsule with a sinker was chosen at random and placed into the dissolution medium. The basket containing the capsules was rotated at a speed of 50 rotations per minute (rpm) to ensure proper mixing of the dissolution medium. A 10 mL aliquot of the sample was taken out at regular intervals (5, 15, 20, 30, 45, and 60 minutes), and the volume withdrawn was replaced with the same volume of 0.1M HCL. The replacement process was carried out to keep the sink in working condition. The samples were filtered and analyzed with the HPLC method. Then the dissolution profile of the developed capsules in 0.1M HCL was obtained by plotting a graph of cumulative drug released against time [22].

Stability Study of Hard Gelatin Capsules

A stability study was conducted to assess the changes in the quality and performance of hard gelatin capsules containing PK-II over a period of six months. The study was conducted in accordance with the guidelines provided by the International Council for Harmonization ICH Q1A (R2). The PK II capsules were kept in a stability chamber (Thermolab) maintained at 40°C±2°C and 75%±5%RH. The acceptance criteria of stability study, including general appearance, assay and dissolution testing, was realized immediately after initial and then after 1,2 ,3 and 6 months of storage in stability chamber. Samples were collected and evaluated at the end of the first, second, third and six months of the stability study. During the study the chamber were observed thoroughly for the temperature and humidity change occurred, if any. [23].

Instrumentation and chromatographic conditions

An Agilent HPLC system (1260 Infinity) was used for the analysis of PK-II. The system was equipped with a G1329B auto sampler system. G1315F variable wavelength detector (Agilent Technologies) was used for the analysis. The chromatographic separation was carried out using an Agilent Phe-Hex column (100 x 4.6 mm, 2.7 µm) analytical column. Isocratic separation was performed using a mixture of 0.1 Formic acid in water: ACN (80:20 v/v) as the mobile phase. Flow rate of the mobile phase was maintained at 1.2 ml/min throughout the separation. The separation was carried out at 400C column temperature. The detection was carried out using a UV at 266 nm. Open Lab EZchrome software was applied for instrumental control, data acquisition and quantitative analysis.

RESULTS AND DISCUSSION

Optimization and Validation of HPLC method

HPLC method for estimation of PK-II in capsule formulation was developed according to the ICH guidelines (table 3 & figure 1). This HPLC method was then validated for linearity, accuracy, precision, range, robustness, limit of detection (LOD), and limit of quantification (LOQ) as per the ICH Q2 (R1) guidelines (ICH Q2 guidelines, 2005).

Table 3. HPLC Conditions for PK II

Sr. No	Parameter	HPLC
1	Instrument	Agilent 1260 Infinity
2	Column	Phe-Hex, 100 X 4.6 mm, 2.7 µm Poroshell 120 Agilent, Column Oven Temp: 40°c
2	Mobile Phase	0.1 Formic acid in water: ACN (80:20 v/v)
3	Flow rate & Run Time	1.2 ml/min & 10 min
4	Detection wavelength	266 nm

6	RT	3.513 min
7	Linearity & Range	> 0.973 & 1 to 25 µg/ml
8	Accuracy (%RSD)	0.6721 to 1.213
9	Precision (%RSD)	0.2621 to 0.9846
10	Robustness study (%RSD)	0.5128 to 0.1.3712
11	LOD & LOQ	0.2 µg/mL & 0.1 µg/ml

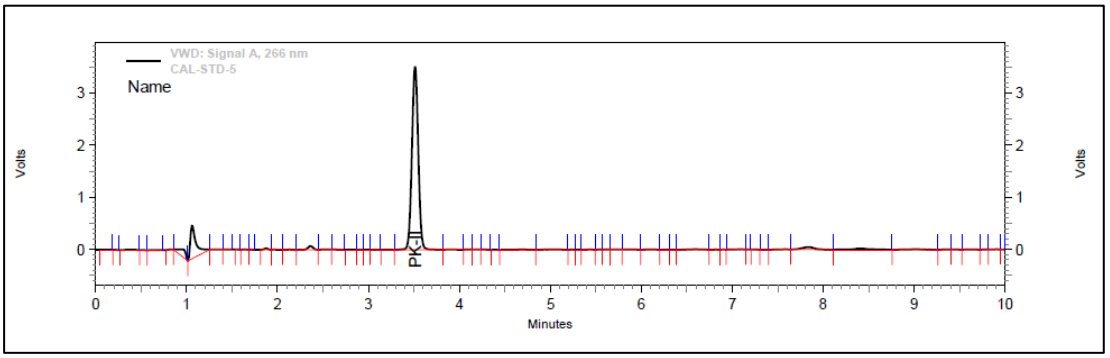

Figure 1. HPLC Chromograph of PK II

Flow Properties of Dried Extract and Granules

The most popular methods for determining flowability are flow rate through an orifice, Hausner's ratio, compressibility index, angle of repose, and others. The powdered formulation had favorable flow characteristics, with an angle of repose of 40.82 ±0.16 and Hausner's ratios and Carr's index of 1.18 ± 0:08% and 39 ± 0:05%, respectively shown in table 4. Powered with good flow characteristics will fill capsules more evenly and make encapsulation easier.

Evaluation of capsule

General appearance

The capsules formulated with PK II exhibited a satisfactory general appearance upon visual inspection. The capsules were meticulously examined for any irregularities such as pinholes, deformities, and overall texture. This examination was conducted under appropriate lighting conditions. No noticeable defects were observed in the capsules' shells, and they appeared to possess a consistent texture and structure. The capsules exhibited a uniform appearance across the batches, suggesting a successful formulation process that maintained the integrity of the capsule shells.

Weight variation analysis

Weight variation test of twenty capsules were carried out. From the results, it was declared that, from the average weight no capsule was deviated by higher than 7.5 %. It was also found that all capsules were passed the weight variation test. The obtained values for each batch are summarized in table 4.

D. Drug content and disintegration test

The assessment of the active ingredient content in the PK II capsules was conducted to ensure the consistency and accuracy of the formulation. A representative sample consisting of ten randomly selected capsules was subjected to analysis. The capsules were carefully opened, and their contents were extracted and transformed into a powdered form using manual techniques. Around 10 capsule of the resultant powder was meticulously dissolved in 10 ml of methanol to form a solution. The solution was then subjected to filtration using PTFE Syringe filter to remove any particulate matter. Filtered samples were analyzed for the PK-II content using proposed HPLC method. The obtained peak areas were fitted in respective calibration curves and concentrations were determined. The obtained values for each batch are summarized in table 4.

Table 4. Pre & post-compression parameters of powder blend

Batch Code	Angle of repose* (θ)	Carr's Index* (%)	Hausner's ratio*	Weight variation analysis	%Drug content	Disintegration time	% CDR
PK-1	32.69 ± 0.19	31.09 ± 0.21	1.18 ± 0.08	6.30 ± 0.10	97.90 ± 0.18	5.26 ± 0.21	98.95 ± 0.13
PK -2	36.43 ± 0.21	33.16 ± 0.12	1.19 ± 0.07	5.08 ± 0.15	81.45 ± 0.29	6.16 ± 0.37	82.54 ± 0.28
PK -3	36.95 ± 0.16	24.87 ± 0.18	1.08 ± 0.09	4.06 ± 0.08	94.19 ± 0.32	4.48 ± 0.41	95.91 ± 0.12
PK -4	29.89 ± 0.10	26.94 ± 0.21	1.22 ± 0.05	5.08 ± 0.10	99.61 ± 0.18	4.25 ± 0.24	98.16 ± 0.12
PK -5	33.78 ± 0.08	39.38 ± 0.11	1.17 ± 0.11	4.06 ± 0.14	95.38 ± 0.19	5.98 ± 0.19	96.83 ± 0.18
PK -6	31.77 ± 0.19	29.02 ± 0.21	1.09 ± 0.09	5.59 ± 0.07	89.14 ± 0.42	7.37 ± 0.22	88.41 ± 0.27
PK -7	36.43 ± 0.17	32.13 ± 0.17	1.24 ± 0.10	3.45 ± 0.09	99.57 ± 0.24	4.34 ± 0.35	99.75 ± 0.18
PK -8	38.97 ± 0.22	34.20 ± 0.13	1.11 ± 0.08	5.99 ± 0.10	96.78 ± 0.27	5.18 ± 0.17	97.58 ± 0.27
PK -9	34.32 ± 0.25	35.24 ± 0.19	1.17 ± 0.05	6.30 ± 0.14	98.61 ± 031	5.55 ± 0.25	98.16 ± 0.37
PK -10	34.57 ± 0.17	29.02 ± 0.20	1.22 ± 0.09	6.10 ± 0.12	93.88 ± 0.22	7.62 ± 0.28	94.80 ± 0.24
PK -11	38.66 ± 0.20	30.05 ± 0.14	1.16 ± 0.04	3.05 ± 0.11	96.81 ± 0.16	5.34 ± 0.21	97.01 ± 0.14
PK -12	37.46 ± 0.18	27.98 ± 0.12	1.25 ± 0.08	2.84 ± 0.11	97.78 ± 0.12	5.88 ± 0.30	97.80 ± 0.14
PK -13	32.69 ± 0.21	25.91 ± 0.27	1.26 ± 0.10	4.98 ± 0.12	96.72 ± 0.35	5.95 ± 0.41	97.27 ± 0.17
PK -14	26.38 ± 0.19	34.20 ± 0.21	2.26 ± 0.05	5.08 ± 0.10	96.78 ± 0.24	5.07 ± 0.18	97.80 ± 0.15
PK -15	24.65 ± 0.15	33.16 ± 0.23	1.17 ± 0.11	6.20 ± 0.08	96.74 ± 0.15	5.78 ± 0.38	97.67 ± 0.23
PK -16	34.47 ± 0.17	31.09 ± 0.13	1.22 ± 0.15	6.40 ± 0.12	96.58 ± 0.19	5.48 ± 0.21	97.85 ± 0.18
PK -17	25.52 ± 0.12	29.02 ± 0.18	1.15 ± 0.09	4.57 ± 0.08	97.54 ± 0.42	4.55 ± 0.26	97.45 ± 0.13
PK -18	26.02 ± 0.10	25.91 ± 0.11	1.17 ± 0.04	3.86 ± 0.09	91.83 ± 0.39	7.68 ± 0.31	92.38 ± 0.15
PK -19	27.77 ± 0.17	31.09 ± 0.15	1.18 ± 0.08	4.98 ± 0.15	95.97 ± 0.28	5.85 ± 0.29	96.79 ± 0.20

PK -20	32.59 ± 0.19	25.91 ± 0.24	1.34 ± 0.10	5.28 ± 0.18	84.01 ± 0.34	6.48 ± 0.20	83.91 ± 0.19
PK -21	26.20 ± 0.21	27.98 ± 0.12	1.33 ± 0.12	4.78 ± 0.14	98.27 ± 0.24	4.79 ± 0.32	99.79 ± 0.18
PK -22	32.69 ± 0.26	32.13 ± 0.15	1.36 ± 0.07	5.89 ± 0.12	87.14 ± 0.29	5.28 ± 0.37	98.47 ± 0.15
PK -23	36.43 ± 0.23	35.24 ± 0.17	1.29 ± 0.05	3.96. ± 0.09	93.84 ± 0.19	7.38 ± 0.24	94.58 ± 0.23
PK -24	33.24 ± 0.18	30.05 ± 0.20	1.33 ± 0.04	5.59 ± 0.15	95.12 ± 0.23	4.88 ± 0.30	96.52 ± 0.19
PK -25	34.56 ± 0.16	27.98 ± 0.26	1.38 ± 0.09	4.88 ± 0.13	96.72 ± 0.37	5.18 ± 0.27	97.27 ± 0.23
PK -26	32.91 ± 0.19	296.39 ± 0.19	1.34 ± 0.07	5.59 ± 0.11	98.94 ± 0.21	4.99 ± 0.19	99.70 ± 0.31
PK -27	28.06 ± 0.10	32.13 ± 0.21	1.34 ± 0.11	5.08 ± 0.07	95.19 ± 0.36	6.18 ± 0.32	95.91 ± 0.38
PK -28	31.34 ± 0.23	31.09 ± 0.18	1.33 ± 0.10	3.86 ± 0.09	94.11 ± 0.40	6.17 ± 0.25	95.51 ± 0.19
PK -29	29.72 ± 0.17	29.02 ± 0.22	1.36 ± 0.05	5.18 ± 0.12	83.28 ± 0.29	7.39 ± 0.20	84.48 ± 0.20
PK-II-OB	32.69 ± 0.20	31.09 ± 0.19	1.18 ± 0.07	6.30 ± 0.10	98.52 ± 0.45%	5.22± 0.37	99.79 ±0.84

***In-Vitro* Dissolution Release**

In vitro drug release studies of the PK II capsule were conducted for a period of one hour. According to the experimental design batches i.e., batch-1 to batch-29 (figure 2) the drug release behavior of each batch was studied. The percent release was obtained in the range of 82.54 % to 99.79% (table 4) which is variable due to the different composition of the Capsule.

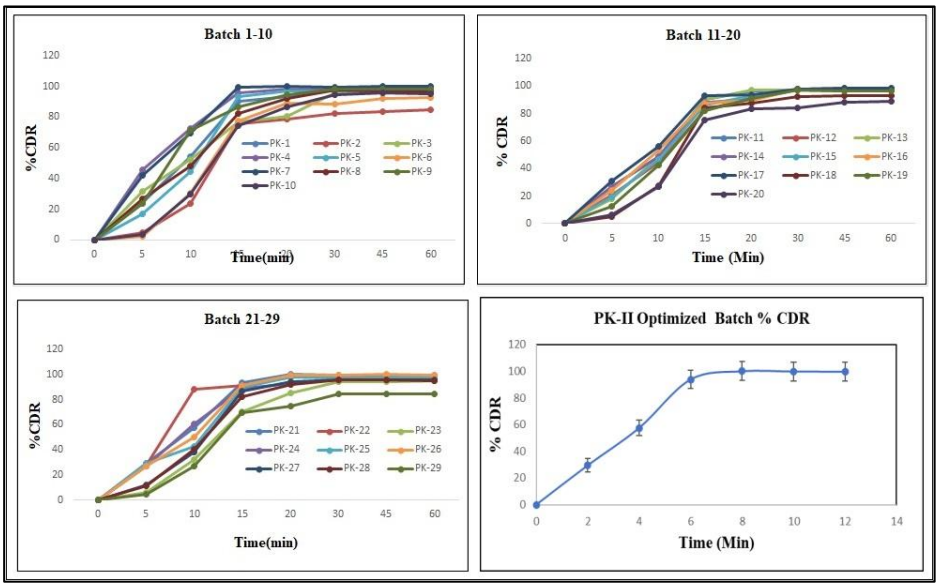

Figure 2. Dissolution Profile of Batch PK-1 to PK-29 & Optimized batch

Characterization and Evaluation of PK II Capsule Formulation

Differential Scanning Calorimetry (DSC) Analysis for PK II:

The DSC analysis of PK II revealed distinct thermal events that provide insights into its thermal behavior and characteristics. The DSC thermogram exhibited a sharp endothermic peak observed at 129^{0}C (figure 3) indicating the melting of PK II. The DSC analysis was effective in characterizing the thermal behavior and stability of the PK II formulation. The observed melting point, crystallization behavior, and thermal transitions contributed to a comprehensive understanding of the formulation's solid-state properties and its response to temperature changes.

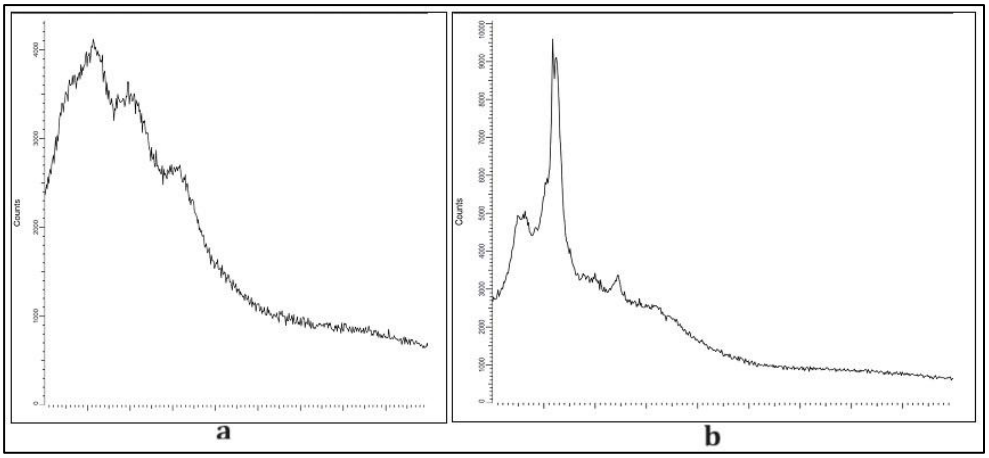

Figure 3. Differential Scanning Colorimetry (DSC) of PK II (a) and Formulation (b)

Fourier Transform Infrared Spectroscopy (FTIR) Analysis for PK II

From the FT-IR spectra of the PK-II (figure 4(a)), and optimized batch A (figure 4(b)) it was observed that, there was no interaction occurs between the entrapped drug and other ingredients

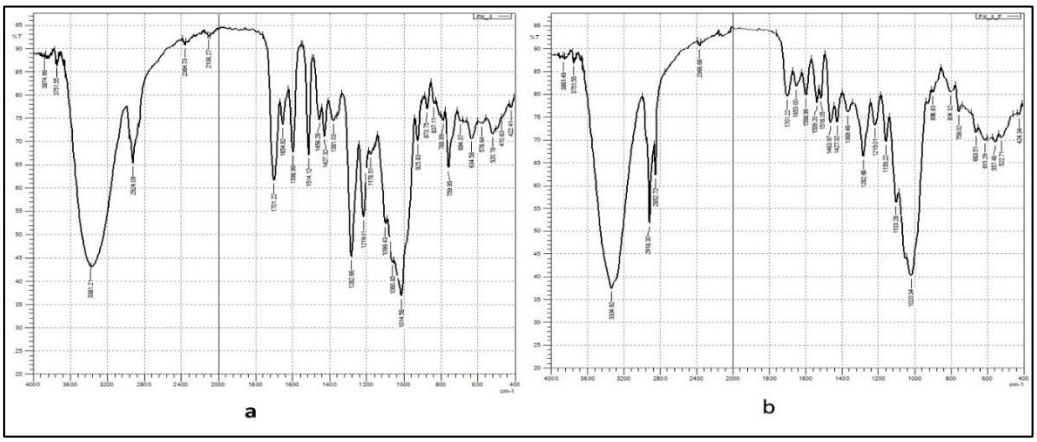

Figure 4. FT-IR analysis of PK II (a) and Formulation (b)

Drug-Excipient Compatibility Study by FTIR

The outcomes of the drug-excipient compatibility study were discerned from a meticulous analysis of the FTIR spectra. Comparison between the spectra of the individual excipients and PK II with those of the physical mixtures revealed crucial insights. Consequently, based on the highly detailed and intricate analysis of the FTIR results, it can be conclusively established that there were no substantial chemical interactions between PK II and the examined excipients. This verdict affirms the safety and compatibility of Magnesium Stearate, MCC, SLS, and Sodium Starch Glycolate as constituents of the PK II capsule formulation (figure 5). The FTIR findings underscore the confidence in utilizing these excipients to encapsulate PK II, ensuring the preservation of its pharmaceutical attributes and therapeutic efficacy in the finalized product

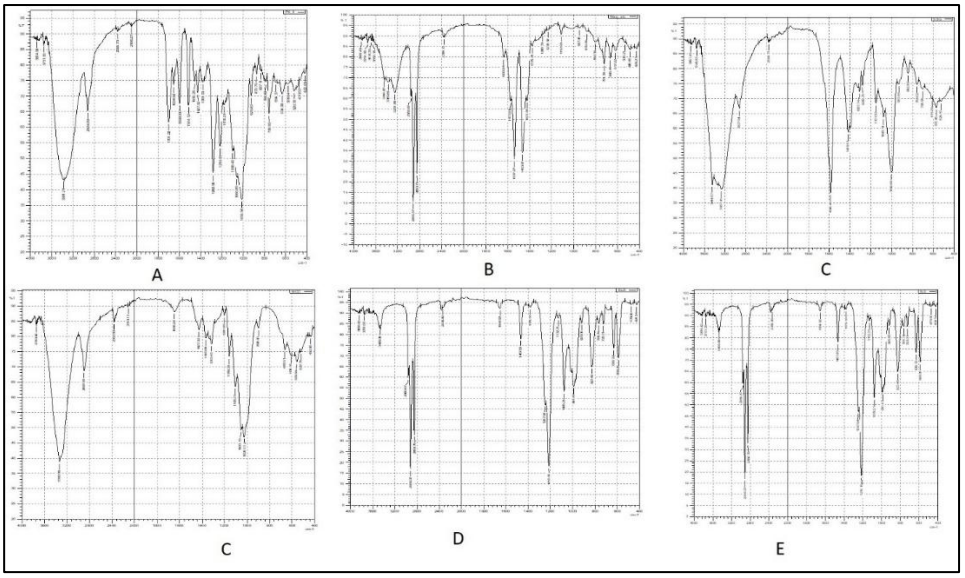

Figure 5. Drug-Excipient Compatibility Study by FTIR (A: PK II, B: Magnesium stearate, C: MCC, D: Sodium Starch Glycolate, E: SLS

Incorporating DOE Statistics Data (ANOVA):

The experimental design based on the Box-Behnken design was rigorously analyzed using Design-Expert software. The statistical analysis, including analysis of variance (ANOVA), revealed the significant effects of the different formulation factors and their interactions on the responses of interest. The software-generated response surface plots and contour plots visually depicted these relationships, aiding in the interpretation of the data.

The statistical analysis yielded valuable insights into the formulation factors that significantly influenced the characteristics of the PK II capsules. These insights guided the selection of an optimized formulation that met the predefined target specifications for various attributes. The incorporation of Design-Expert software's desirability function facilitated the systematic determination of the optimal formulation that achieved a balance between multiple responses.

The percent drug release was selected as the response of the PK II capsule formulation. The mathematical model describing the percent drug release of PK II as functions of the coded independent variables table 5 in the selected range was demonstrated by the following second order polynomial equations.

R1=+67.56932+0.467639A+5.22721B+4.29236C-7.71807D-0.027549AB-0.023929AC +0.0990 00AD-0.287368BC-0.261053BD-0.073333CD-0.002534A2-0.202992B2-0.151806C2 +0.650000D2

The significance of each coefficient was determined using p-value, which is used as a tool to check the interaction strength between each independent variable. When a factor and an interaction among variables have a p- value less than 0.05, they influence the process in a significant way for a confidence level of 95%. The significance of the F- value depends on the analysis of variance table 5 showed that this regression model was highly significant ($p < 0.01$) with F- values of 57.47 and 54.82 for percent drug release and total drug content respectively. The F- values of 394.60 and 383.43 for lack of fit implies that they are not significant comparing to the pure error.

Table 5. ANOVA for PK II formulation determined from Box–Behnken experimental design

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	549.57	14	39.26	7.44	0.0003	**significant**
A-Microcrystalline Cellulose	311.10	1	311.10	58.95	< 0.0001	
B-Magnesium Stearate	45.28	1	45.28	8.58	0.0110	
C-Sodium Starch Glycolate	38.09	1	38.09	7.22	0.0177	

D-Sodium Lauryl Sulphate	5.67	1	5.67	1.07	0.3174	
AB	20.98	1	20.98	3.98	0.0660	
AC	25.25	1	25.25	4.78	0.0462	
AD	12.01	1	12.01	2.28	0.1537	
BC	16.77	1	16.77	3.18	0.0963	
BD	0.3844	1	0.3844	0.0728	0.7912	
CD	0.0484	1	0.0484	0.0092	0.9251	
A^2	62.49	1	62.49	11.84	0.0040	
B^2	8.50	1	8.50	1.61	0.2250	
C^2	12.11	1	12.11	2.29	0.1521	
D^2	0.1713	1	0.1713	0.0325	0.8596	
Residual	73.88	14	5.28			
Lack of Fit	72.04	10	7.20	15.63	0.0087	**significant**
Pure Error	1.84	4	0.4608			
Cor Total	623.45	28				

Significant at p ≤ 0.0500; Not significant at p ≥ 0.001; NS: not significant

The regression equation is graphically represented by the 3D response surface plot and the outcomes of the percent drug release as influenced by the percentage of Microcrystalline Cellulose, Magnesium Stearate, Sodium Starch Glycolate and Sodium Lauryl Sulphate shown in figure 6. The response surface curve plot shown in figure 6(a) represents the relationship between microcrystalline cellulose and magnesium stearate, this response plot shows the effect of Magnesium Stearate and Microcrystalline Cellulose on drug release. When concentration of Magnesium Stearate and Microcrystalline Cellulose increased, drug release increased. Microcrystalline Cellulose is used as Diluents in formulation, its show effect on drug release of drug. 6 (b) represents the relationship between microcrystalline cellulose and sodium starch glycolate, this response plot shows the effect of Sodium Starch Glycolate and Microcrystalline Cellulose on drug release. When concentration of Sodium Starch Glycolate and Microcrystalline Cellulose increased, drug release increased. Sodium Starch Glycolate is used as Disintegrate in formulation, its show effect on drug release of drug. 6 (c) represents the relationship between microcrystalline cellulose and sodium lauryl sulphate, this response plot shows the effect of Sodium lauryl sulfate and Microcrystalline Cellulose on drug release. When concentration of Microcrystalline Cellulose increased, drug release increased and there is no impact on release of Sodium lauryl sulphate concentration. 6 (d) represents the relationship between sodium starch glycolate and magnesium stearate, this response plot shows the effect of Magnesium Stearate and Sodium Starch Glycolate on drug release. When concentration of Magnesium Stearate and Sodium Starch Glycolate increased there is slightly increase in drug release.

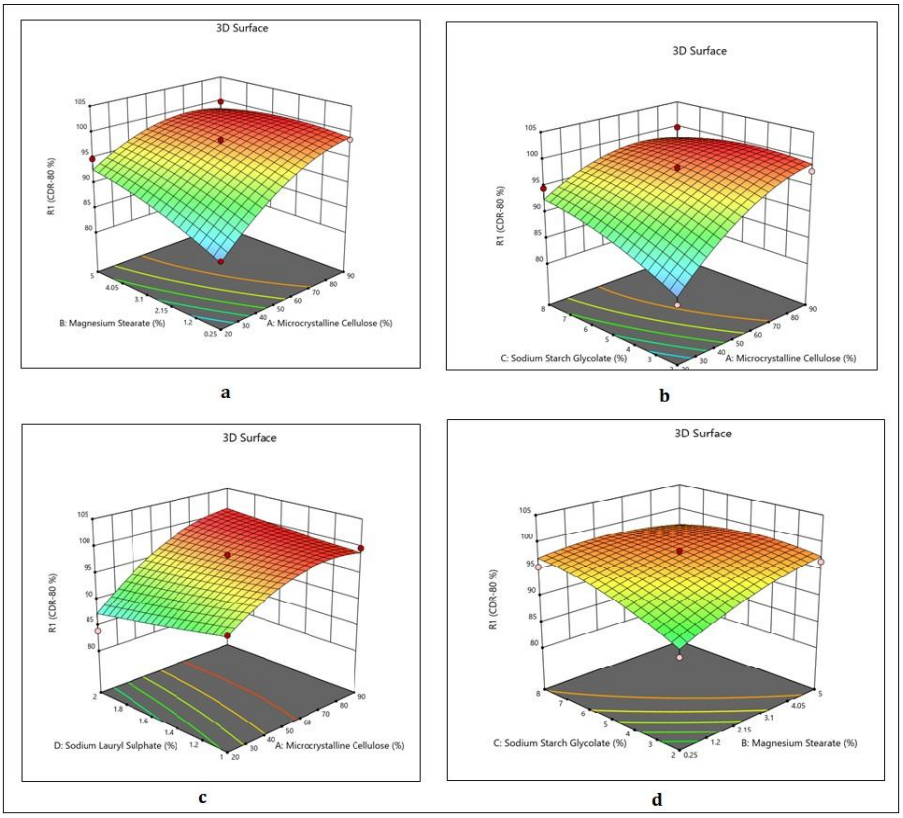

Figure 6. 3D Response surface plots showing the effects of variables on the percent release of PK II

This response plot shows predicted batches given by DE optimized batch (figure7 & table 6). The above graph indicates that all the points are close to centre line so we concluded that actual result and predicted result are very close to each other. We obtained a certain point in the graph at which achieved optimum result, ahead of which any increase in concentration of polymer had no effect on drug release.

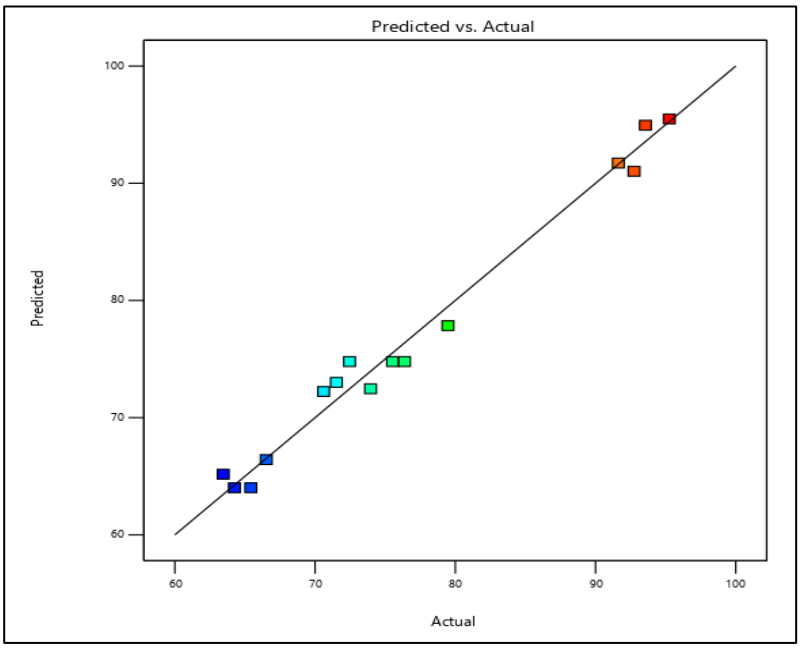

Figure 7. Predicted batches given by DE Optimized batch

Table 6. Optimized Batch by Software

Batch	MCC Conc. (%)	Mag. Stearate Conc. (%)	SSG Conc. (%)	SLS conc. (%)
Optimized Batch	90	2.625	5	1

Stability Study of Hard Gelatin Capsules

Throughout the six-month stability study, the hard gelatin capsules containing PK-II were subjected to the specified temperature and humidity conditions. At the end of the study period, samples from each batch were retrieved for evaluation. The appearance of the capsules was inspected, and no significant changes were noted in terms of color, texture, or visible defects. The stability study of capsule after each month was given in table 7.

Table 7. Stability study of PK II capsule formulation

Test	Proposed Specification	Initial	One Month	Two Month	Three Month	Six Month
General Appearance	Consistent fine texture and structure	Consistent fine texture and structure	Consistent fine texture and structure	Consistent fine texture and structure	Consistent fine texture and structure	Consistent fine texture and structure
Assay Label Claim (20mg/ capsule)	Between 95.0 to 105.0% of label claim	99.70 %	99.20 %	98.59	98.12%	98.05%

3. CONCLUSION

More and more, people are turning to herbal medications manufactured from plant extracts to treat a broad range of clinical illnesses, and scientists are working hard to figure out how these treatments work. Many people who suffer from liver illness use herbs. Separating the medicines' actual therapeutic benefit from the unfounded hopes linked with them will require future efforts to employ substantial methodological advances. To make the agents suitable for therapeutic usage, it is necessary to extract the active molecules and then test them in well-designed tests and, lastly, randomized, placebo-controlled trials.

The present research work reports the development of oral capsule formulation of picroside II. The optimized formulation of the picroside II showed good micromeritic properties with enhances drug content, entrapment efficiency and stability issues. In-vitro release study showed significant increase in the drug release profile of the optimized batch. Hence it can be concluded that employing QbD approach in the formulation of PK-II capsules has leaded to a pharmaceutically equivalent, low cost, quality improved and stable formulation.

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5. CONFLICTS OF INTEREST

The authors declare no conflict of interest

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