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Original Article

Microwave-Assisted Nanocomposites for Solubility Enhancement of Azelnidipine: Development, Optimization and *in vivo* Pharmacokinetic Study in Rats

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ABSTRACT

Background: The enhancement of drug solubility and bioavailability for orally administered medications remains a significant challenge in pharmaceutical formulations. This study focused on Azelnidipine, a poorly water-soluble drug used to treat hypertension, aiming to improve its solubility and delivery using microwave-assisted nanocomposite technology. **Materials and Methods:** A 3^2 factorial design was utilized to optimize the concentrations of polymers HPMC K100M and PVK K-30 in the nanocomposite formulation. The optimization was carried out using Design-Expert® software with a quadratic model validated by ANOVA, ensuring statistical significance. Physical characterization of the polymers, including swelling index and FTIR spectroscopy, was conducted to determine their suitability as carriers. *In vitro* drug release studies and *in vivo* pharmacokinetic studies were performed, with *in vivo* experiments conducted on Wistar rats (*n*=6 per group) to compare the optimized nanocomposite (AF6) with a marketed product. **Results:** The optimized nanocomposite displayed a significant improvement in solubility, with the highest solubility observed at 1.685 mg/mL (AF6) and the highest drug content at 91.76% (AF6). *In vivo* studies demonstrated a peak plasma concentration of 1450.33 ng/mL for AF6, markedly higher than the marketed product's peak of 850 ng/mL. AF6 maintained elevated plasma concentrations over 12 hr and showed a superior AUC of 8608.58 ng/mLh compared to 2818.58 ng/mLh for the marketed product. **Conclusion:** The study successfully employed microwave-assisted nanocomposite technology to enhance the solubility and bioavailability of Azelnidipine. The optimized nanocomposite Formulation (AF6) significantly outperformed the marketed product in terms of drug content, solubility and pharmacokinetic profile, offering a promising approach to improving oral drug delivery for poorly soluble drugs.

Keywords: Azelnidipine, Solubility enhancement, Microwave-assisted nanocomposites, *In vivo* pharmacokinetics, Nanocomposite.

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INTRODUCTION

The oral route of administration remains the most preferred and convenient method for drug delivery due to its ease of administration, patient compliance and flexibility in dosage form design.[1,2] This pathway facilitates the systemic absorption of drugs through the gastrointestinal tract, making it essential for pharmaceutical compounds to possess adequate solubility and stability in the digestive system.[3,4] However, the development of oral medications faces numerous challenges, particularly with drugs that exhibit poor water solubility. Such drugs are often associated with low bioavailability, limited therapeutic efficacy and variable pharmacokinetic profiles, necessitating innovative strategies to enhance their solubility and overall performance in oral formulations.[5-7]

Solubility enhancement techniques have emerged as pivotal in the pharmaceutical field to address the solubility challenges of poorly water-soluble drugs.[8] Among these drugs, Azelnidipine, a dihydropyridine calcium channel blocker used in the treatment of hypertension, exemplifies the solubility-related challenges that can hinder its therapeutic effectiveness and bioavailability when administered orally.[9,10] Azelnidipine's poor aqueous solubility and consequent low oral bioavailability necessitate the exploration of novel solubility enhancement methods.[11,12] The development of microwave-assisted nanocomposites presents a promising approach to overcoming these hurdles. This innovative technique leverages the energy of microwaves to fabricate nanocomposite materials that can significantly improve the solubility and, ultimately, the bioavailability of Azelnidipine, offering a potential pathway to enhance its clinical efficacy and patient outcomes in hypertension management.[13,14]

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Nanocomposites, engineered materials composed of nanoparticles integrated into a matrix, have gained substantial attention in pharmaceutical research for their potential to improve drug solubility and bioavailability. These materials can alter the physical and chemical properties of Active Pharmaceutical Ingredients (APIs), such as Azelnidipine, by enhancing their dissolution rate and stability.[15] Microwave-assisted synthesis stands out as a cutting-edge technique in the fabrication of nanocomposites, offering advantages over traditional methods including reduced reaction times, lower energy consumption and improved product purity.[16] This approach utilizes microwave radiation to induce rapid heating, enabling the uniform distribution of nanoparticles within the composite material. The controlled environment provided by microwave synthesis allows for the fine-tuning of nanocomposite properties, such as particle size, morphology and crystallinity, which are crucial for optimizing drug solubility and delivery.[17]Top of Form

The primary objective of this study is to develop and characterize microwave-assisted nanocomposites specifically designed to enhance the solubility of Azelnidipine, addressing a critical challenge in its oral administration. By leveraging the unique properties of nanocomposites synthesized through microwave-assisted techniques, this research aims to significantly improve the dissolution rate and bioavailability of Azelnidipine, potentially leading to enhanced therapeutic efficacy in the management of hypertension. Furthermore, this study seeks to comprehensively evaluate the developed nanocomposites through various characterization techniques, including particle size analysis to elucidate their physicochemical properties and solubility enhancement mechanisms. Lastly, an *in vivo* pharmacokinetic study in rats will be conducted to assess the bioavailability of Azelnidipine when delivered via the developed nanocomposites, compared to its conventional formulations. Through these objectives, the study aims to provide a foundational understanding of microwave-assisted nanocomposites as a viable solubility enhancement strategy for poorly water-soluble drugs like Azelnidipine, contributing valuable insights to the field of drug delivery and formulation science.

MATERIALS AND METHODS

Materials

The Azelnidipine was procured as gift from Sciquaint Innovations (OPC) Private Limited, Pune, India. The Hydroxy Propyl Methyl Cellulose (HPMC) was procured from Research Lab Fine Chem Industries, Mumbai, India, PVK30 were purchased from Meru Chem Pvt. Ltd., Mumbai, India, all analytical grade chemicals and solvents were employed.

Methods

Calibration curve of Azelnidipine

The calibration curve for Azelnidipine was validated following the International Council for Harmonisation (ICH) guidelines. Azelnidipine 50 mg was dissolved in phosphate buffer pH 6.8 and the volume was then adjusted to 100 mL with water to create a stock solution.[17] After that, this solution was diluted in phosphate buffer pH 6.8 to produce standards of 1, 2, 3, 4, 5 and 6 ppm. Using a pH 6.8 phosphate buffer as a blank, the absorbance of these standards was measured at 284 nm using a UV spectrophotometer. This proved that UV spectrophotometry at λ_{max} of 284 nm is a suitable technique for figuring out the quantities of azelnidipine in aqueous solutions.[18]

Determination of Solubility

The solubility of Azelnidipine was determined by dissolving an excess amount of the drug in 10 mL of distilled water and phosphate buffer at pH 6.8. This mixture was continuously agitated for 24 hr at 37°C to ensure complete dissolution. Afterward, the solution was allowed to equilibrate. The solution was then filtered through a membrane filter to remove any undissolved particles. The solubility of the filtrate was analyzed using a UV-visible spectrophotometer, specifically the Shimadzu UV-2600 model.[19]

Drug- Excipient compatibility Studies.

Compatibility studies were conducted to ensure there were no interactions between the drug and excipients in the formulation. The drug and its physical mixtures with the polymer (in a 1:1 ratio) were placed in sealed vials and stored in a desiccator. The storage conditions in the desiccator were carefully controlled, maintaining a temperature of 25°C±2°C and a relative humidity of 0% (using anhydrous calcium chloride as the desiccant) for one month. After the storage period, FTIR spectra were recorded using a Shimadzu IR Affinity-1S spectrometer. The spectral data were analyzed using OriginPro v.9.2 software, which facilitated the identification and comparison of characteristic peaks to evaluate the compatibility of Azelnidipine with the excipients.[19]

Experimental Design for Nanocomposite

Nine batches of nanocomposite formulations were prepared following a 3^2-factorial design, using the qualitative factors and levels presented in Tables 1 and 2. The experimental design was generated and evaluated with Design Expert software Expert® DX 13.0 (StatEase Inc., MN). Two independent variables were examined: the amount of HPMC K100M (A) and PVK K-30 (B). The dependent variables selected for evaluation were drug content (R1) and solubility (R2).[20]

Preparation Method for Physical Mixture in Nanocomposites

To prepare the nanocomposites, a precise amount of the drug was mixed homogeneously with the polymer in weight-to-weight (w/w) with different concentration as detailed in Table 1, maintaining a constant mixture volume. For each gram of polymer, 4 mL of water was added to the drug-polymer mixture to form uniform slurry. This slurry was then placed in a round-bottom flask and exposed to microwave radiation at 556 W for 5 min, with continuous stirring. The resulting nanocomposites were then ground and sieved to achieve a particle size distribution of 80 to 250 µm. The nanocomposites utilizing HPMC K100M and PVK k-30 polymers.[21]

Evaluation of Nanocomposites

Solubility Study

The solubility of physical mixes and Nanocomposites (NCs) in pH 6.8 phosphate buffer was determined using a conventional method. Based on the solubility data, the drug-to-carrier ratio was optimized in order to achieve the best results.[22]

Drug content analysis

Drug content analysis was carried out to ascertain the quantity of drug integrated into the nanocomposites. We dissolved the produced nanocomposites in 25 mL of phosphate buffer with a pH of 6.8. After passing through a 0.45 µm membrane filter, the resultant solution was examined at a wavelength of 284 nm with a UV-visible spectrophotometer. For accuracy, the outcomes were compared to a blank solution of pH 6.8 phosphate buffer.[22]

Particle size analysis

The nanocomposite sample weighing 0.5 g was diluted using 10 mL of double-distilled water. Next, a laser scattering particle size analyzer (Malvern Particle Analyzer) was used to evaluate the sample's particle size and zeta potential.[23]

Stability study of optimized nanocomposites

The International Council for Harmonization's (ICH) guidelines were followed in the performance of an accelerated stability study. For three months, the optimized nanocomposite sample was kept in a stability chamber with a temperature of 40±2°C and a relative humidity of 75±5%. Throughout the stability research, assessments of the medication's appearance, content and *in vitro* drug release were carried out once a month for one, two and three months.[24]

Physical Characterization of Polymer

Swelling Index (SI)

The method for calculating the swelling index of polymers was modified. Accurate measurements of 1 mg of HPMC K4M and PVK K-30 were made and the results were transferred to a 100 mL measuring cylinder. It was noted how much initial volume the polymer occupied. After that, distilled water was used to precisely adjust the volume to 100 mL. After covering the cylinder's open end with aluminum foil, it was left alone for a full day. Following this time frame, the enlarged polymer's volume was determined. The following formula was used to determine each polymer's swelling index.[24]

$$SI = \frac{Hf - Hi}{Hi} \times 100 \quad (1)$$

Where, SI-Swelling index of gum,

Hi-Initial height of powder,

Hf-Final height of powder after 24 hr.

In vivo Pharmacokinetic Study

The procedure was carried out in accordance with the Acute Toxic Class Method, previously detailed in the Chen F. *et al.*[25] Animals were categorized into four groups: Group I (Control), Group II (Nanocomposite (AF5)), Group III (Marketed Product-Azeldip 16), Prior to the start of the study and during its course, the animals were subjected to a fasting period of 12 hr, with water being freely available.[26] All studies were conducted post-receipt of institutional animal ethical clearance (Approval No. 1942/PO/Re/S/17/CPCSEA/2022/01/09).

The rats were given a dose of 0.51 mg/kg body weight through oral gavage with the help of a feeding tube. For pharmacokinetic analysis, blood samples were taken at different intervals (0, 1, 2, 3, 4, 5, 6-, 7-, 8- and 12-hr after dosage). Heparinized tubes containing blood were obtained from the retro-orbital plexus and centrifuged at 3,000 rpm for 10 min at room temperature. High-performance liquid chromatography was used to ascertain the drug's Plasma Concentration (HPLC). The M-721 system controller, the M-730 data module, the M-501 solvent delivery pump, the WISP-712 autosampler and the M-481 variable wavelength UV detector made up the HPLC system. On a symmetrical C18 stainless steel column (150 mm×3.9 mm inner diameter, 5 µm), chromatographic separations were performed. The mobile phase was conducted at a flow rate of 1.3 mL/min and contained 0.01 M potassium phosphate, acetonitrile and methanol (70:20:10% v/v/v) with a pH set to 6.7. At 284 nm, the detection was carried out at a chart speed of 0.5 cm/min and a sensitivity of 0.01 absorbance units full scale.[26]

Statistical analysis

The data were analyzed using GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA, USA) and the Student's *t*-test. The findings are shown as mean (±SD). Design Expert software version 13.0 was used for formulation optimization. All statistical analyses were conducted with a significance threshold of 0.05. PK Solver Software was used to analyze pharmacokinetic parameters for various non-compartmental Pharmacokinetic (PK)

parameters, such as Area Under the Curve (AUC), Area under the first Moment Curve (AUMC), maximum plasma Concentration (C_{max}), time to reach C_{max} (T_{max}) and Mean Residence Time (MRT). In addition, one-way Analysis of Variance (ANOVA) and the Dunnett post hoc test were used to statistically assess the PK data for multiple comparisons.

RESULTS

Physical Characterization of Polymers

Physical characterization of both polymers, HPMC K100M and PVK k-30, revealed different swelling, viscosity and foaming properties. The swelling capacity of PVK k-30 was found to be better than HPMC K100M as 92.52±3.45% was found as compared to 88.14±2.45% of HPMC K100M. Nevertheless, HPMC K100M had higher viscosity values of 8.69±0.89 cp compared to 6.74±1.07 cp for PVK k-30, suggesting that it can make more viscous solutions. Compared to PVK k-30 (6±0.93), HPMC K100M had a higher foaming index (8±0.78) indicating that it had better surface active properties. These also would imply that these two polymers would function well in combination, yielding these distinct physical properties that would indicate complementary formulation characteristics. Based on their higher swelling capacity and superior viscosity, PVK k-30 and HPMC K100M could have a synergistic effect on the control of drug release and formulation stability.

Drug Content Analysis

The nanocomposite batches (AF1-AF9) reported by the drug content analysis were found to have notable variations. Batch AF1 had the lowest drug content of 75.82±0.57% and batch AF6 the highest drug content of 91.76±0.11%. Except for AF6, there was a general increasing trend of drug incorporation from AF1 to AF6. Reciprocal results were found for the remaining ones (AF7-AF9) in which the drug content of the batches was kept within the range of 81–83%. However, the difference in drug content between different batches could be due to variations in polymer concentration and processing parameters during nanocomposite preparation.

Solubility Analysis

The solubility studies revealed large variations in solubility between different nanocomposite formulations. The highest solubility exhibited was 1.685±0.07 mg/mL for Batch AF6, 1.564±0.04 mg/mL for Batch AF8, which is a dramatic improvement over the others. AF1 (0.151±0.09 mg/mL) showed the lowest solubility, where the formulation parameters were found to have had significant influence on the solubility enhancement. Notably, batches AF5 and AF7 displayed moderate solubility improved values of 0.663±0.01 and 0.695±0.09 mg/mL, respectively. This variation in solubility across batches indicates that polymer composition and processing parameters had important effects on drug solubility profile.

Particle Size Analysis

Particle size analysis showed that all the batches of nanocomposite were successfully prepared in having size between 166.3 to 193.5 nm. Batch AF5 (166.3±0.67 nm) and AF9 (169.2±0.44 nm) produced particles with the smallest size. Batch AF3 (193.5±0.73 nm) were found to deposit the largest particles. All batches

Table 1: Factors and Levels for the 3^2 Factorial Design.

Label	Factors	Level (mg)		
		Low (-)	Medium	High (+)
A	HPMC K100M (mg)	400	800	1200
B	PVK k-30 (mg)	100	200	300

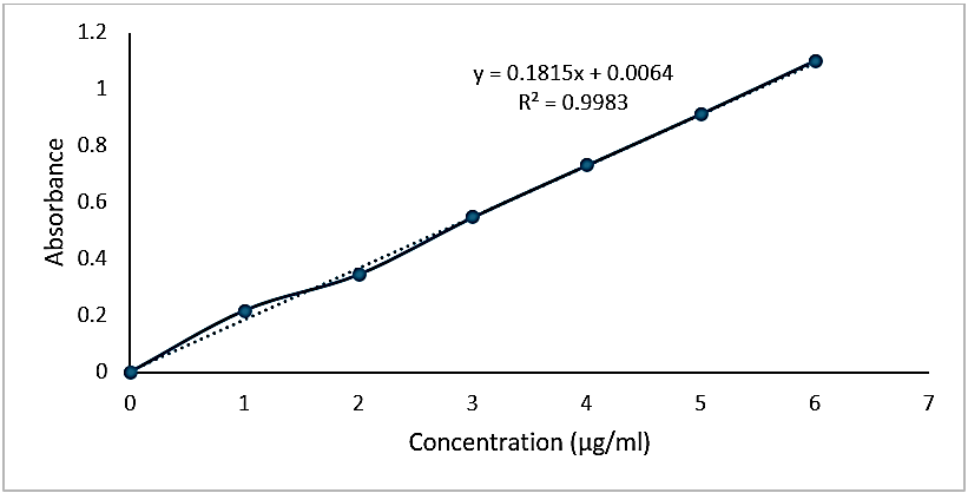

Figure 1: Calibration curve of Azelnidipine in Phosphate Buffer pH 6.8.

exhibit good control over the particle size during the formulation process, as seen from the relatively narrow range of particle sizes. The particle size most formulations could be held below 190 nm, an advantageous characteristic for enhanced absorption and bioavailability of the drug.

Results of Fourier-Transform Infrared Spectroscopy (FTIR)

The infrared spectroscopy analysis detailed in Figure 2 highlights a promising compatibility between Azelnidipine and the excipients HPMC and Povidone K-30, essential for solubility enhancement formulations. Notably, the N-H stretch of Azelnidipine at 3333.22 cm^{-1} showing a minimal shift to 3334.76 cm^{-1} in the physical mixture, alongside the C-H stretch moving slightly from 2972.53 cm^{-1} to 2974.73 cm^{-1}, indicates that the primary structural and hydrogen bonding characteristics of Azelnidipine remain virtually unchanged. The C=O stretch frequency's consistency at 1708 cm^{-1} across pure and mixed samples further underscores the intactness of crucial functional groups responsible for the drug's activity. Although there are variations in the aromatic C=C stretch (from 1597.56 cm^{-1} in Azelnidipine to ranges between 1599.62, 1579.27, 1507.44 and 1473.33 cm^{-1} in the mixture) and the C-O stretch (shift from 1212.37 cm^{-1} in Azelnidipine to 1204.66 and 1214.53 cm^{-1} in the mixture), these modifications suggest minor alterations in the electronic environment rather than detrimental interactions.

Optimization of the Concentrations of HPMC K100M and PVK k-30 using 3^2 Factorial design

ANOVA for Quadratic model for Drug Content (R1)

The analysis of drug content optimization using statistical parameters revealed the model fitness with the significant value (p=0.0195) for Table 3. The regression equation (2) for drug content is shown to give linear effects of HPMC K100M (A) (2.22), of PVK K 30 (B) (1.19) and their interaction (AB) (-1.89) on drug content. A significant curvature in the response surface was evidenced by the negative exponent of the quadratic term B^2 (-7.30). Figure 3 (1a) and (1b) clearly visualize this situation, where the 2d contour and 3d surface plot shows how variations in polymer concentrations have rises with respectively increasing concentrations of both polymers to a certain point, afterwards such increase is in decline and sometimes even goes in a decrease. The higher F-value (17.77) and lower p-value (0.0195) of the model indicate its significance. Moreover, the F value of the B^2 term was highest (59.21, p=0.0046) of all terms, indicating that the quadratic PVK k-30 concentration was the most influential variable in drug content.

The regression equation obtained for Drug Content is as follows:

$$\text{Drug Content} = +88.84 + 2.22^{*}A + 1.19^{*}B - 1.89^{*}AB - 0.6900A2 - 7.30^{*}B2 \quad \ldots\ldots\ldots\ldots (2)$$

ANOVA for Quadratic model for Solubility (R2)

As shown in Table 3, the solubility optimization model showed high significance (p = 0.0044) and high F value (49.65). The regression equation (3) reveals interesting relationships between

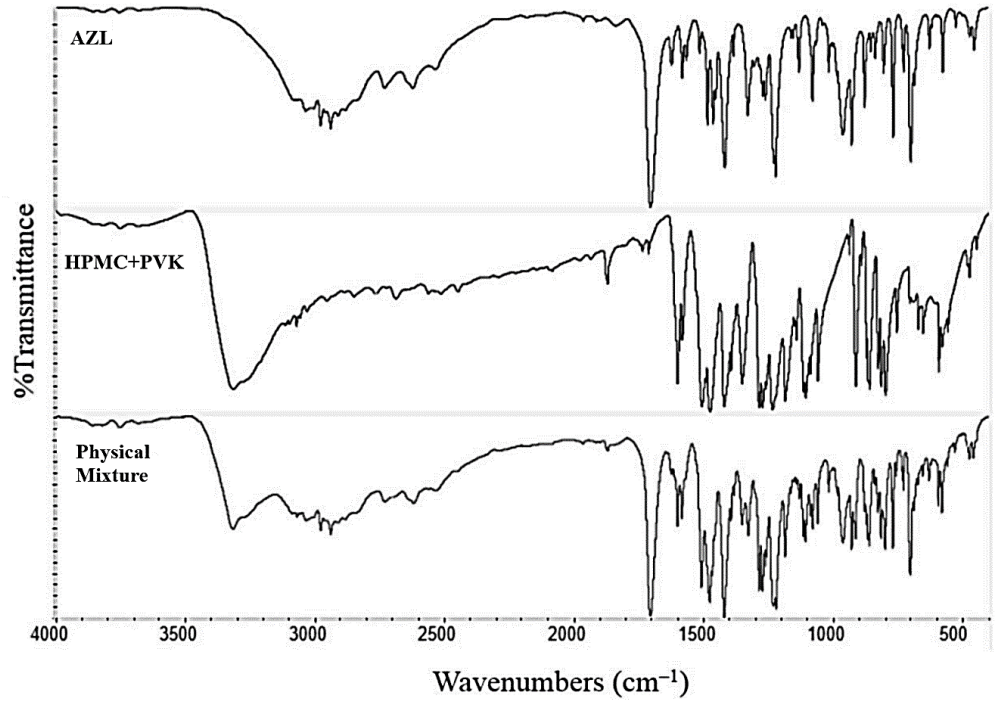

Figure 2: Overplay plot of FTIR spectra of Drug, HPMC+PVK k-30 and Physical mixture.

Table 2: Preparation of Nanocomposites batches using 3^2 factorial designs.

Ingredients (mg)	AF1	AF2	AF3	AF4	AF5	AF6	AF7	AF8	AF9
Azelnidipine	500	500	500	500	500	500	500	500	500
HPMC K100M	400	800	1200	400	800	1200	400	800	1200
PVK k-30	100	100	100	200	200	200	300	300	300

Table 3: Summary of ANOVA for Response Surface Quadratic Models.

Source	Sum of squares	Degree of freedom	Mean square	F-value	*P*-value
Drug Content (%)					
Model	159.685	5	31.94	17.77	0.0195
A-HPMC K100M	29.57	1	29.57	16.45	0.0270
B-PVK k-30	8.47	1	8.47	4.71	0.1183
AB	14.25	1	14.25	7.93	0.0670
A^2	0.9522	1	0.9522	0.5297	0.5194
B^2	106.43	1	106.43	59.21	0.0046
Solubility (mg/mL)					
Model	2.31	5	0.4615	49.65	0.0044
A-HPMC K100M	0.0032	1	0.0032	0.3465	0.5975
B-PVK k-30	0.3015	1	0.3015	32.44	0.0107
AB	0.0262	1	0.0262	2.82	0.1915
A^2	1.78	1	1.78	191.42	0.0008
B^2	0.1974	1	0.1974	21.24	0.0192

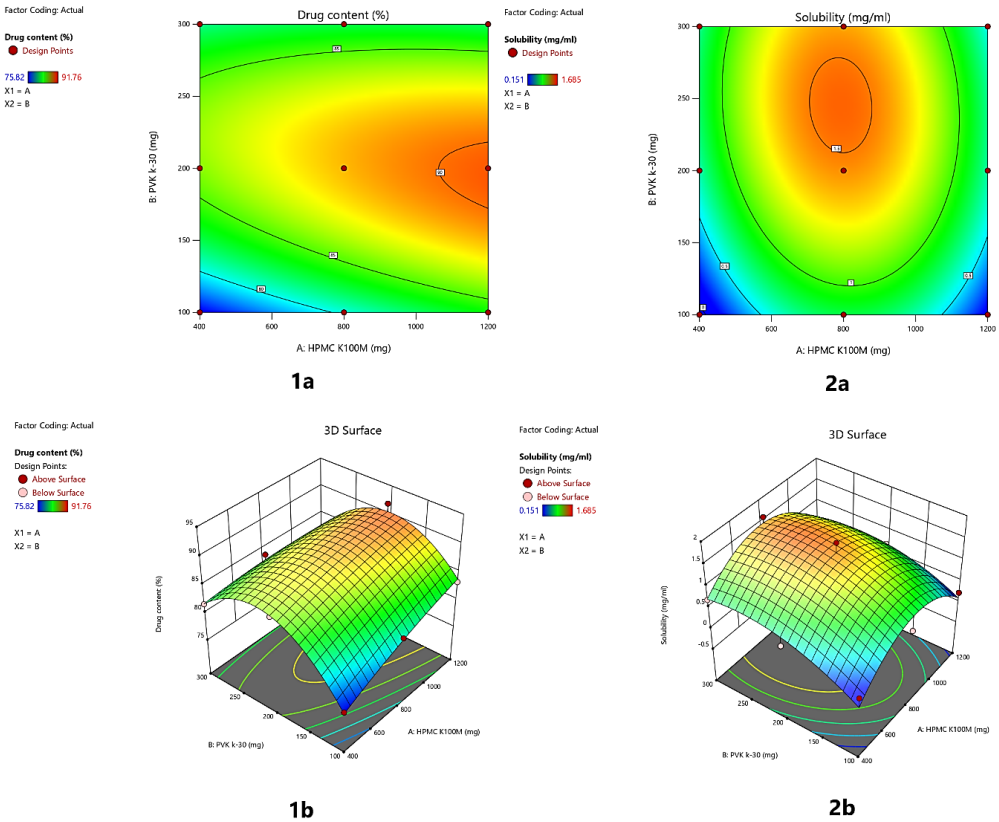

Figure 3: 2D Contour (1a) and 3D surface (1b) plots illustrating the effect of HPMC K100M and PVK K-30 on the drug content of the nanocomposite. 2D Contour (2a) and 3D surface (2b) plots represent the influence of HPMC K100M and PVK K-30 on the solubility of the nanocomposite.

polymers and solubility. PVK k-30 (B) had a positive linear term coefficient (0.2242) whereas the effect of HPMC K100M (A) was a slightly negative one (-0.0232). Negative coefficients of both quadratic terms (A^2 with coefficient -0.9432; B^2 with -0.3142) show an optimal concentration above which solubility decreases. This peak of solubility at optimal polymer concentrations is clearly illustrated in Figure 3 (2a) and (2b); where the contour and 3D surface plots are involved. The quadratic term A^2 (the highest F value (191.42, p=0.0008)) and the linear effect of PVK k-30 (F value = 32.44, p=0.0107) were the most significant factors affecting solubility. The interaction term (AB) had a relatively small significance level (F-value=2.82, p=0.1915), implying that the individual polymer concentrations played a primary role in determining solubility more than the interaction of polymer concentrations.

The regression equation obtained for Solubility (R2) is as follows:

$$\text{Solubility}=+1.62 -0.0232*A +0.2242*B -0.0810*AB -0.9432A2 -0.3142*B2 \quad (3)$$

Validation of Statistical Model

The predicted drug content and solubility parameters of the optimized formulation (AF6) compared well to the experiment (Table 4). There is a minimal percentage error of -1.373% with a predicted value of -90.387% and an experimental drug content of 91.76%. The predicted value (0.662 mg/mL) was similar to the experimental solubility (0.663 mg/mL), indicating the reliability and accuracy of the optimization process.

In vitro drug release studies

In vitro drug release profiles for nanocomposite formulations AF1 through AF9 over a 12 hr period (Figure 6), showing a consistent, time-dependent increase in drug release. All formulations start with no release, but by the 1-hour point, AF6 leads with a notably higher release (10.89%). This trend persists, with AF6 consistently showing the highest release at subsequent time points, peaking at 90.04% at 12 hr, closely followed by AF9 at 89.33%. Other formulations also demonstrate significant release rates, though less than AF6 and AF9, with AF5 and AF8 showing notable cumulative release percentages above 67% at the 10-hour mark.

Stability Study Analysis

Table 5 shows the stability study the optimized formulation (AF6) performed over three months at 40±2°C and 75±5% RH, which showed excellent stability characteristics. They kept their physical appearance good throughout the course of the study. Minimal degradation of the drug content was observed with less than 90.67±1.78 to 89.43±2.98% after three months. The values of

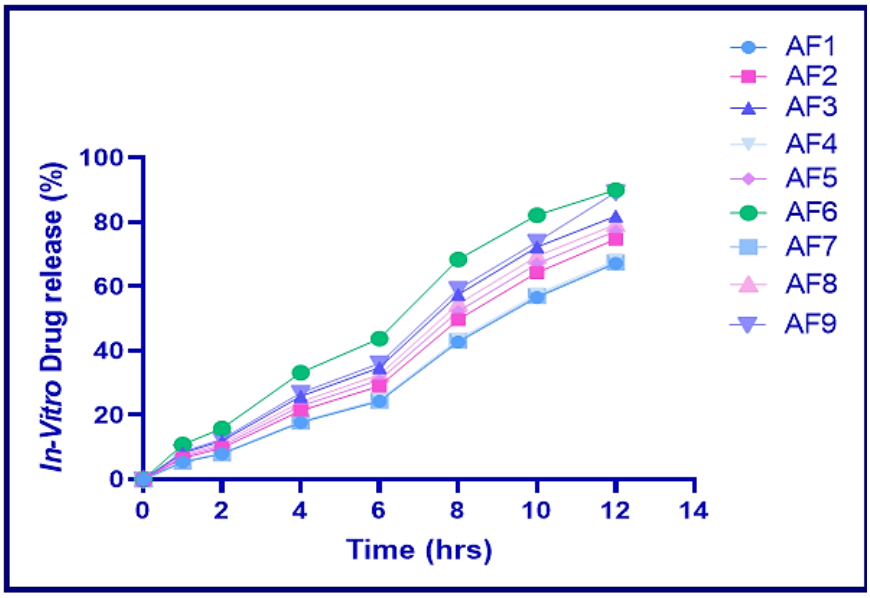

Figure 4: *In vitro* drug release studies of Nanocomposite.

Table 4: The predicted and experimental values of response variables, along with the corresponding percentage error, are compared.

F. Code	Composition (%w/v)	Response	Predicted value	Experimental value	Percentage Error
AF6	HPMC K100M	Drug Content	90.387	91.76	-1.373
	PVK k-30				
AF6	HPMC K100M	Solubility	0.662	0.663	-1.373
	PVK k-30				

solubility were stable between 0.897±0.06 mg/mL and 0.893±0.09 mg/mL. Formulation stability under accelerated conditions was achieved and drug release at 12 h exhibited remarkable consistency throughout the study period with values between 91.76±0.64% and 92.67±0.69% and providing a proof of stability.

Pharmacokinetic study

Table 6 shows the in vivo pharmacokinetic study of the optimized nanocomposite (AF6) over marketed product. AF6 showed superior performance as demonstrated in the study. The peak plasma concentration over the admission period was noted to be significantly higher with nanocomposite formulation (4 hr 1450.21±98.81 ng/mL) as compared to the marketed product (950±123.72 ng/mL). In addition, the optimized formulation showed significant improvements in pharmacokinetic parameters presented in Table 7, such as a higher AUC (8608.58±241 ng/mLh vs. 2818.58±617 ng/mLh), similar T_{max} (4 h), similar C_{max} (1450.33±7.25 ng/mL vs 850±17.25 ng/mL), and longer Mean Residence Time (3.697±6.2 h vs 2.62±3.31 h). These results also show the development of the nanocomposite formulation, which confers enhanced bioavailability and prolonged therapeutic effect over the conventional marketed product.

DISCUSSION

The calibration curve of Azelnidipine, represented in Figure 1, demonstrates a strong linear relationship between the drug concentration and its absorbance at 284 nm, with a range from 0.1 to 1.102 µg/mL. The graphical representation in Figure 1 confirms the linear progression, crucial for pharmacokinetic studies where precise measurement of drug concentrations over time is paramount.

The physical characterization of HPMC K100M and PVK K-30, as shown in Table 6, including their swelling index, viscosity and foaming index, aligns well with previous findings. The swelling index of HPMC K100M was 88.14±2.45%, consistent with values reported by Sonawane *et al.*,[21] who observed a similar swelling index for HPMC in nanocomposite formulations, indicating its high-water absorption capacity beneficial for controlled release. Similarly, the swelling index of PVK K-30 was slightly higher at 92.52±3.45%, which is in agreement with the findings of Bergese *et al.*[14] who noted that PVK K-30 exhibits rapid swelling, making it suitable for immediate-release formulations.

The viscosity measurements also corroborate with literature, where HPMC K100M exhibited a viscosity of 8.69±0.89 cp, falling within the range observed by Sonawane *et al.*,[21] who reported viscosities between 8 and 10 cp for HPMC. PVK K-30's viscosity of 6.74±1.07 cp aligns with the findings of Bergese *et al.*[14] supporting its use in formulations where lower viscosity is preferred. The foaming index for HPMC K100M was 8±0.78, consistent with the moderate foaming properties reported by Sonawane *et al.*[21] while PVK K-30 had a lower foaming index of 6±0.93, aligning with its expected performance in minimizing unwanted foaming in drug delivery systems, as discussed by Bergese *et al.*[14] These comparisons validate the suitability of both polymers in the nanocomposite formulations developed in this study.

The infrared spectroscopy analysis detailed in Figure 2 highlights a promising compatibility between Azelnidipine and the excipients HPMC and Povidone K-30, essential for solubility enhancement formulations. The presence of new peaks such as C-H bending and aromatic out-of-plane vibrations in the mixture (at 910, 857 and 754.43 cm^{-1}, respectively) indicates the successful integration of excipients without compromising Azelnidipine's structural integrity. This analysis collectively demonstrates a lack of significant interaction between Azelnidipine and the excipients, supporting their suitability for enhancing the drug's solubility and maintaining its pharmacological efficacy.

The solubility different batches of nanocomposite formulations (AF1 to AF9) reveal a significant variation in drug content, solubility and particle size, underscoring the impact of formulation and process parameters on nanocomposite performance. Notably, batch AF5 stands out with the highest solubility of 1.685 mg/mL and a relatively smaller particle size of 166.3 nm, suggesting a strong correlation between reduced particle size and enhanced solubility, likely due to the increased surface area available for dissolution. Conversely, batches like AF1 and AF3, with lower solubility values (0.151 mg/mL and 0.225 mg/mL, respectively) and larger particle sizes indicate that not all formulations achieve optimal solubility enhancement. The drug content across batches varies, with AF6 showing the highest value at 91.76%, which, despite not having the highest solubility, underscores the importance of drug loading efficiency in formulation performance. Batch AF8 also demonstrates significant solubility improvement (1.564 mg/mL) with a modest particle size (176.1 nm), highlighting that alongside particle size, other formulation factors such as polymer matrix composition and drug-polymer interactions play crucial roles in solubilizing the drug.

The optimization of the concentrations of HPMC K100M and PVK K-30 using a 3^2 factorial design, as depicted in Table 3 and Figure 3, demonstrates a significant impact on the drug content of the nanocomposite, a critical parameter for ensuring therapeutic efficacy. The Analysis of Variance (ANOVA) for the quadratic model reveals that the model is significant, with an F-value of 17.77 and a *p*-value of 0.0195, indicating that the regression model reliably predicts the variation in drug content based on the concentrations of HPMC K100M and PVK K-30. The individual contributions of HPMC K100M (A) and PVK K-30 (B) to the drug content are evident from their F-values and *p*-values. HPMC K100M shows a more substantial influence (F-value of 16.45 and *p*-value of 0.0270) compared to PVK K-30 (F-value of 4.71, *p*-value of 0.1183), suggesting that HPMC K100M's concentration is a

Results Stability study

Table 5: The result of a stability study on various parameters of the optimized batch (AF6). The temperature is maintained at 40±2°C and the relative humidity is kept at 75± 5%.

Response	0	1 month	2 months	3 months
Appearance	Good	Good	Good	Good
Drug Content (%)	90.67±1.78	90.53±0.68	90.32±0.77	89.43±2.98
Solubility (mg/mL)	0.897±0.06	0.789±0.08	0.778±0.04	0.893±0.09
Drug Release at 12 hr (%)	91.76±0.64	91.43±0.96	91.88±0.79	92.67±0.69

Data is expressed in mean±SD, (*n*=3).

Results of *In vivo* Pharmacokinetic Study

Table 6: Mean plasma concentration results for the optimized Nanocomposite and a batch of the Marketed product.

Time (hrs)	Nanocomposite (AF6)	Marketed Product
0	0	0
1	869.66±85.21	419.37±51.59
2	1216.42±64.69	600.31±53.59
4	1450.21±98.81	950±123.72
6	1343.33±97.51	461.33±107.61
8	898±65.5	319.66±96
12	603.33±38.52	113.33±14.57

*All values are expressed as mean±SD, (*n*=3).

Table 7: Results of pharmacokinetic parameters.

PK parameters	Nanocomposite (AF6)	Marketed Product
AUC (ng/mL*h)	8608.58±241	2818.58±617
T_{max} (h)	4	4
C_{max} (ng/mL)	1450.33±7.25	850±17.25
AUMC (ng. h2/mL)	31830±0666	7388.41±710
MRT (h)	3.697±6.2	2.62±3.31

*All values are expressed as mean±SD, (*n*=3).

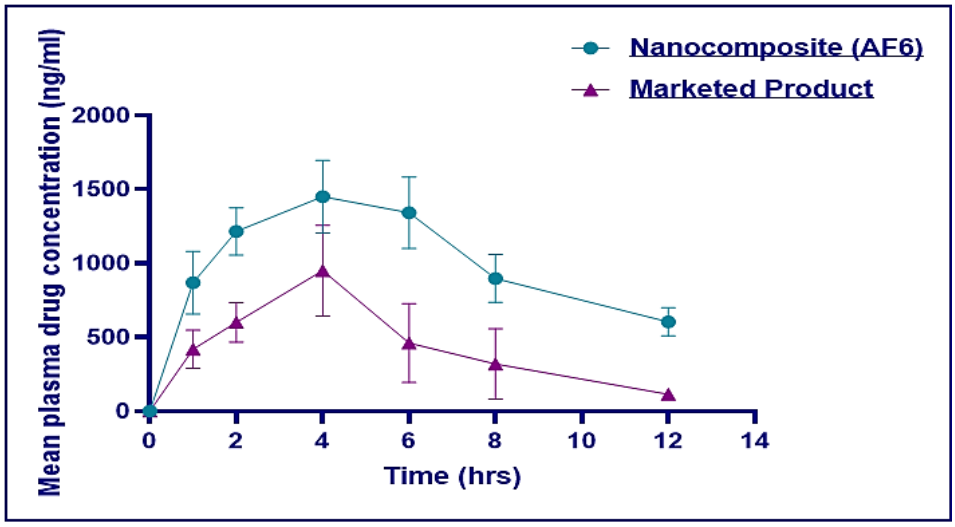

Figure 5: The plasma concentration versus time profiles following administration of the Optimized batch of Nanocomposite and Marketed product.

more critical factor in enhancing drug content. The interaction term (AB) also demonstrates a significant effect (*p*-value of 0.0670), indicating that the interaction between HPMC K100M and PVK K-30 affects the drug content, although to a lesser extent than their individual contributions. Interestingly, the quadratic terms for HPMC K100M (A^2) and PVK K-30 (B^2) provide further insight. The B^2 term is highly significant (F-value of 59.21, *p*-value of 0.0046), indicating a pronounced quadratic effect of PVK K-30 concentration on drug content, which suggests an optimal concentration range for maximizing drug content.[27] In contrast, the A^2 term is not significant (*p*-value of 0.5194), suggesting that the effect of HPMC K100M concentration on drug content does not follow a quadratic relationship within the studied range. Figure 3 (1a and 1b) succinctly illustrates the relationship between the concentrations of HPMC K100M and PVK K-30 and their impact on the drug content in nanocomposite formulations, as shown by both contour and 3D surface plots. The contour map's color gradient points towards the region of highest drug content, indicated by warmer colors, suggesting an optimal polymer combination range. The 3D plot offers a tangible representation of this relationship, with a discernible peak denoting the most advantageous polymer mix for maximal drug content.

The results from the ANOVA of the quadratic model for solubility (R2) presented in Table 3 and visually interpreted in Figure 3 (2a and 2b); suggest a complex relationship between the concentrations of HPMC K100M and PVK K-30 and the solubility of the nanocomposite. The overall model is significant, indicating that polymer concentration does affect solubility; however, individual effects of HPMC K100M, PVK K-30 and their interaction do not significantly affect solubility on their own, as suggested by the non-significant *p*-values. The observed significance in the quadratic term for HPMC K100M indicates a non-linear relationship, with a potential optimal concentration for maximizing solubility. In contrast, the quadratic effect of PVK K-30 is less pronounced.[28] The contour plot in Figure 3 (2a) highlights the optimal concentration range for both polymers, showing a peak in solubility within a specific bounded region. The 3D plot reaffirms this, suggesting that deviations from this optimal range in either direction could lead to reduced solubility. This data is instrumental for formulation scientists to pinpoint the exact concentrations of polymers needed to optimize the solubility of the nanocomposite, which is a crucial step in developing an effective drug delivery system.

Figure 4 presents *in vitro* drug release profiles for nanocomposite formulations AF1 through AF9 over a 12 hr period, showing a consistent, time-dependent increase in drug release. The data from all batches depict a sustained release pattern, with a pronounced difference between the formulations, particularly AF6 and AF9, which may be attributed to their specific composition or structure that favors quicker dissolution and absorption, potentially beneficial for achieving desired therapeutic effects with controlled release properties[29].

The optimal batch was determined to be AF6 by means of statistical model validation. The great accuracy of the model is shown by comparing the experimental values for Drug Content and Solubility with the values predicted by the Design-Expert program, as shown in Table 4. The formulation process's dependability is further supported by the low standard deviation of Drug Content and Solubility, which validates AF6 as the batch that satisfies the study's optimization requirements.[30] The model's efficacy is demonstrated by the AF6 batch's successful validation as the optimized batch. The stability study of the optimized batch (AF6) under conditions of 40±2°C temperature and 75±5% relative humidity over three months demonstrated consistent results across various parameters (Table 5). The optimized batch (AF6) demonstrated stable drug content, solubility and release profiles over the three-month stability study period.

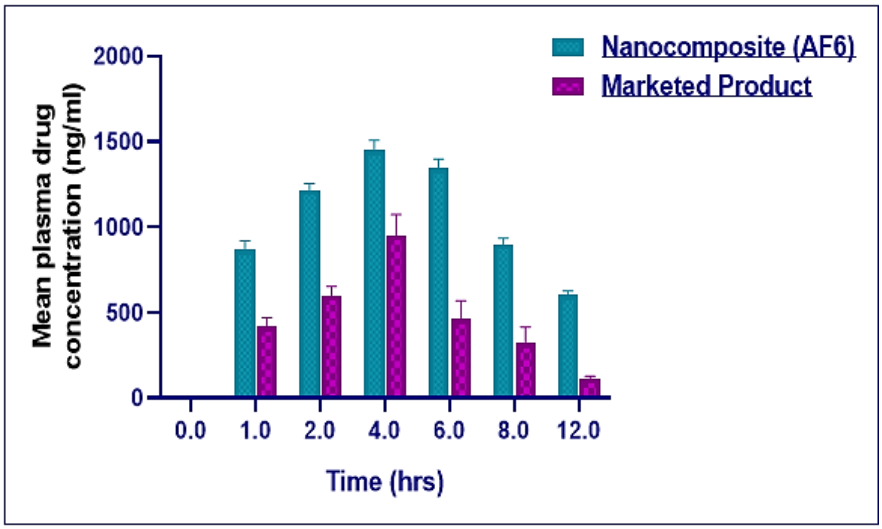

Figure 6: The mean plasma concentration versus time profiles following administration of the Optimized batch of the nanocomposite and Marketed product in bar view.

Figure 7: Dose Administration in Rats and Blood Collection from Retro-Orbital Plexus in Rats.

The *in vivo* pharmacokinetic study of the optimized nanocomposite formulation (AF6) presents a compelling case when compared to the marketed product, as evident from Table 5 and Table 6, graphical representations in Figures 4, 5 and 6. Beginning with the initial plasma concentrations, there is a marked disparity at the first hour post-administration, with the nanocomposite exhibiting a mean concentration of 869.66 ng/mL, more than double that of the marketed product at 419.37 ng/mL. This trend of the nanocomposite achieving higher concentrations continues throughout the study, with a peak Concentration (C_{max}) of 1450.33 ng/mL at 4 hr for AF6, compared to 850 ng/mL for the marketed product, which also peaks at the same time interval, indicating a similar T_{max} for both products. As the study progresses, the nanocomposite maintains superior concentrations up to the 8-hr mark (898 ng/mL compared to 319.66 ng/mL) and concludes with a more pronounced presence at 12 hr (603.33 ng/mL compared to 113.33 ng/mL for the marketed product).[31] The prolonged presence of the drug in the system for the nanocomposite is quantitatively supported by the pharmacokinetic parameter AUC, where AF6 shows an AUC of 8608.58 ng/mLh, significantly outstripping the marketed product's 2818.58 ng/mLh, thereby indicating a more extended exposure and potential therapeutic effect.

The Area under the Moment Curve (AUMC), which is a metric for the total drug exposure and the time the drug, resides in the body, further attests to the enhanced performance of the nanocomposite. AF6 records a higher AUMC of 31830 ng.h^2/mL compared to 7388.41 ng.h^2/mL for the marketed product, suggesting a prolonged drug action.[31] Consistently, the Mean Residence Time (MRT) for AF6 is greater at 3.697 hr than that for the marketed product at 2.62 hr, implying that the nanocomposite formulation retains the drug in the systemic circulation for a longer duration. Figures 5 and 6 visually correlate with the tabulated data, illustrating the higher and sustained plasma levels achieved by the nanocomposite. Figure 6 portrays the plasma concentration-time profile, while Figure 7 provides a comparative bar view representation, both underscoring the nanocomposite's superior performance.

CONCLUSION

The study successfully demonstrated that microwave-assisted nanocomposite technology significantly enhances the solubility and bioavailability of Azelnidipine, a poorly water-soluble drug, thereby confirming the initial hypothesis that this approach could overcome solubility-related challenges in drug delivery. The optimized nanocomposite formulation (AF6) achieved a marked improvement in drug solubility (1.685 mg/mL) and drug content (91.76%), which translated into superior pharmacokinetic performance *in vivo*, with a peak plasma Concentration (Cmax) of 1450.33 ng/mL and an AUC of 8608.58 ng/mL*h, significantly outperforming the marketed product. These results underscore the potential of this technology to enhance the therapeutic efficacy of poorly soluble drugs. Future research should focus on exploring the clinical relevance of these findings, including conducting clinical trials to assess the safety and efficacy of the optimized formulation in humans. Additionally, this technique could be applied to other BCS Class II and IV drugs to address similar solubility challenges, potentially expanding its application across a wider range of pharmaceuticals.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

HPMC: Hydroxypropyl methylcellulose; **UV:** Ultra-Violet; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **IAEC:** Institutional Animal Ethic Committee; **D_f:** Degree of freedom; **RM:** Room Temperature; **AUC:** Area Under Curve; **AUMC:** Area Under the Moment Curve; **MRT:** Mean Residence Time; **PVK:** Polyvinylpyrrolidone; **HPLC:** High-performance liquid chromatography.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Approval of Ethical was obtained from Institutional Animal Ethic Committee (IAEC) (Approval Number: 1942/PO/Re/S/17/CPCSEA/2022/01/09).

SUMMARY

Microwave-assisted nanocomposites were developed and optimized to enhance the solubility and pharmacokinetic profile of Azelnidipine. The study demonstrated a high correlation coefficient (R^2=0.9983) in the calibration curve, indicating precise drug quantification. Characterization of polymers HPMC K100M and PVK K-30 revealed their potential for controlled and immediate-release formulations, respectively. Infrared spectroscopy confirmed the compatibility between Azelnidipine and excipients. Among the nanocomposite batches, AF5 showed the highest solubility, while AF6, optimized through a 3^2 factorial design, exhibited the best drug content and solubility. Stability studies indicated that AF6 maintained consistent performance over three months. *In vivo* pharmacokinetic studies in rats revealed that AF6 achieved significantly higher plasma concentrations and a prolonged drug release profile compared to a marketed product, indicating its potential for improved therapeutic efficacy.

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