RESEARCH ARTICLE

Formulation Design and Development of Azilsartan Nano-emulsion for the Solubility Enhancement

Ravikiran M Suryawanshi*, Ritu M Gilhotra, Prashant Kumar Dhakad, Tapasvi Gupta

Gyan Vihar School of Pharmacy, Suresh Gyan Vihar University, Jaipur, Rajasthan 302017, India

Received: 12th Jan, 2025; Revised: 17th Feb, 2025; Accepted: 4th Mar, 2025; Available Online: 25th Mar, 2025

Abstract

The study aims to formulate and refine a nanoemulsion containing azilsartan to improve the solubility and bioavailability of antihypertensive drug, which has low water solubility. A factorial design technique was employed to develop 14 nanoemulsion formulations with varying amounts of olive oil, Tween 80, and PEG-400. Formulations were analyzed for particle size, Zeta Potential (ZP), pH, viscosity, and drug content. FTIR and DSC studies validated the compatibility of drug and excipient. The improved formulation (F12) demonstrated a mean particle size of 249 nm, a ZP of -22.3 mV, and a drug content of 98.38%. SEM and TEM analysis showed spherical droplets exhibiting smooth surfaces. In vitro release experiments indicated a sustained drug release, with 98.25% released over an 8-hour period. The stability investigations verified that the formulation maintained stability for three months under accelerated situations. Response surface methodology and contour plots indicated a robust design space where formulation parameters could be optimized to achieve desired quality attributes. The nano-emulsion approach successfully improved the aqueous solubility of azilsartan while maintaining physicochemical stability. This novel formulation strategy shows promise for enhancing the therapeutic efficacy of azilsartan and potentially other poorly water-soluble drugs. Further *in vivo* studies are warranted to evaluate the pharmacokinetic profile and bioavailability enhancement of the optimized azilsartan nanoemulsion formulation.

Keywords: Nano-emulsion, Azilsartan, antihypertensive, FTIR, DSC, Solubility Enhancement

How to cite this article: Ravikiran M Suryawanshi, Ritu M Gilhotra, Prashant Kumar Dhakad, Tapasvi Gupta. Formulation Design and Development of Azilsartan Nano-emulsion for the Solubility Enhancement. International Journal of Drug Delivery Technology, 2025;15(1):285-95. doi: 10.25258/ijddt.15.1.40

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Hypertension is still one of the primary global health issues across the world as it affects millions of people and is associated with multiple cardiovascular complications [1]. Effective management of hypertension is needed to lower the risks associated with heart disease, stroke, and other related disorders [2]. Azilsartan has emerged as a promising antihypertensive agent as it is a strong antagonist of angiotensin II receptor and possesses superior efficacy along with a good safety record. Unfortunately, as with many other medications in this class, azilsartan suffers from inadequate solubility in water, thus limiting bioavailability and the drug's therapeutic efficacy [3–5].

The challenge of low solubility has always posed a problem in the development of new drugs, causing researchers to investigate numerous methods dedicated to improving the dissolution and absorption rates of drugs. Amongst these techniques, nano-emulsion technology stands out due to its increased focus and proven capabilities in enhancing both the solubility and bioavailability of drugs [6]. Thermodynamically stable, optically isotropic nano-emulsions are mixtures of oil and water that contain surfactants, with droplet sizes normally varying between 20 to 200 nm [7].

This study primarily targets the design and development of azilsartan nano-emulsion as a novel strategy to cover the solubility issues related to the medicine. The unique, underlying characteristics of nano-emulsions such as their small droplet size, large surface area, and ability to enhance drug permeation through biological membranes are employed in order to enhance the solubility and thus bioavailability of azilsartan [5,8].

The project will include stepwise procedures in oil phase, surfactant and co-surfactant selection for the preparation of nano-emulsions. Different formulation conditions will be refined to produce a stable nano-emulsion system with optimal physicochemical properties. The study will also analyse how the nano-emulsion formulation alters the solubility, dissolution profile, and possible in vitro activity of azilsartan [9,10].

This study aims to advance antihypertensive medication by developing optimized azilsartan nano-emulsions, potentially providing a more effective and patient-oriented treatment for hypertension. The results of the study may facilitate the application of nano-emulsion technology to enhance the solubility of additional therapeutically effective but weakly water-soluble medicines, therefore addressing a significant challenge in drug development [8].

MATERIAL AND METHODS

The Indian company Alembic Pharmaceuticals Pvt. Ltd. supplied the azilsartan. Hi-Media Lab of Mumbai supplied

Author for Correspondence: rkiran091989@gmail.com

Table 1: Variable levels for improvement

Batch-Code	X1:Water(%)	X2:Olive oil(%)	X3:Smix (%)
F-1	0.5	0	0.5
F-2	0.5	0.5	0
F-3	0.333333	0.333333	0.333333
F-4	1	0	0
F-5	0.666667	0.166667	0.166667
F-6	1	0	0
F-7	0.166667	0.666667	0.166667
F-8	0	1	0
F-9	0.5	0.5	0
F-10	0	0	1
F-11	0	0	1
F-12	0	0.5	0.5
F-13	0.166667	0.166667	0.666667
F-14	0	1	0

the pectin. Moychem Pvt. Ltd. provided the olive oil, almond oil, and eucalyptus oil, while Loba Chem. Pvt. Ltd. of Mumbai gave the sodium chloride, ethyl alcohol, chloroform, acetone, potassium dihydrogen phosphate (KH2PO4), and PEG-400 and Tween80. All material was used for analytical grade. Aqueous solvents such as double distilled water were used.

Physical Characterization and Identification

The obtained drug sample was subjected for physical examination and identification by UV, FTIR, DSC, and solubility studies.

Physical Evaluation

Azilsartan is first identified in the pre-formulation research by its physical examination for identification purposes, including its appearance, color, and taste [11].

Determination of Lambda Max and Calibration Curve

A pure 100mg sample of Azilsartan was weighed and then dissolved methanol and the sample was then placed in a sonicator for 10 minutes and the sample solution was

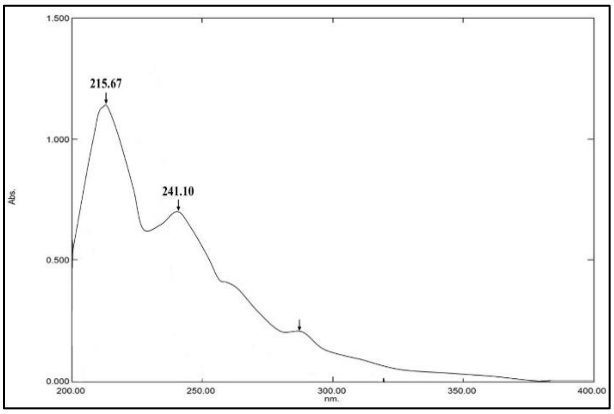

Figure 1: UV spectrum of Azilsartan

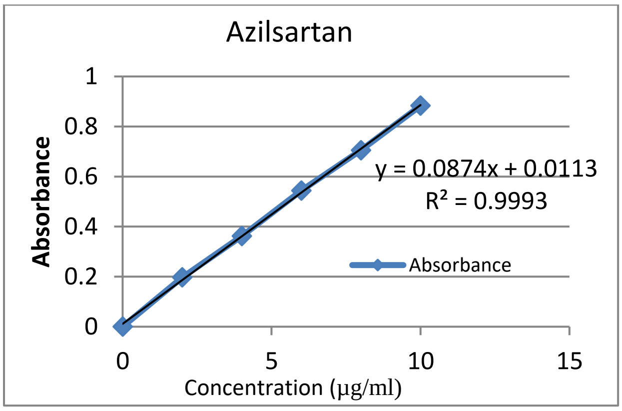

Figure 2: Calibration Curve of Azilsartan

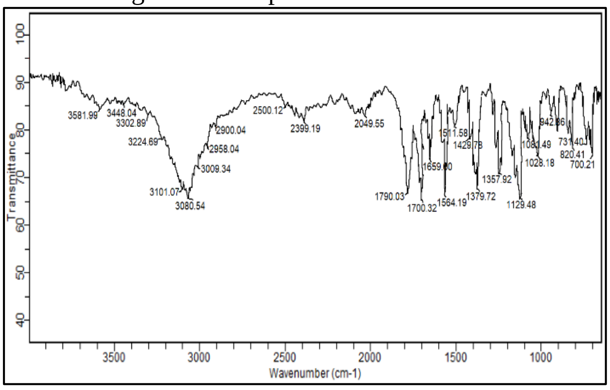

Figure 3: FTIR spectra of Azilsartan

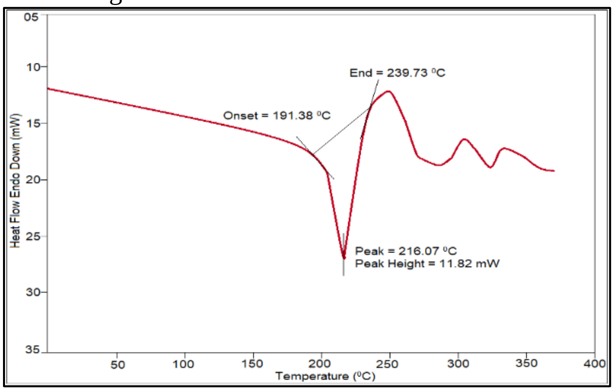

Figure 4: DSC spectra of Azilsartan

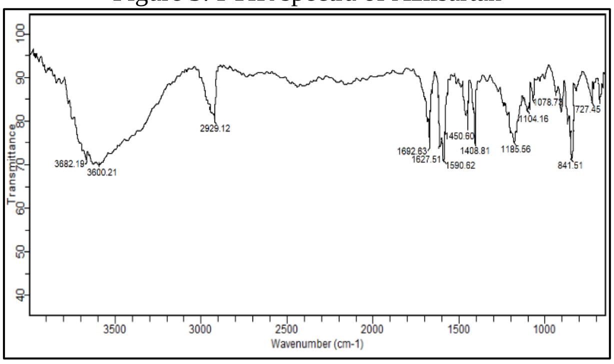

Figure 5: FTIR-spectra of Pectin

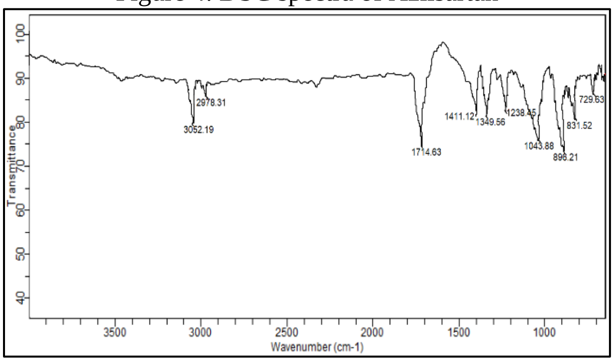

Figure 6: FTIR-spectra of PEG-400

Table 2: Simple Latex Design (SLD)

Batch-Code	X1:Water(%)	X2:Olive oil(%)	X3:S_{mix} (%)	Particle-Size(nm)	ZP (mV)
F-1	62	2	34	251	18
F-2	64	2	32	261	20
F-3	66	2	32	249	30
F-4	63	2	33	257	28
F-5	62.21	2.64	32.16	238	26
F-6	63	2	32	258	25
F-7	62.36	1.32	32.64	270	18
F-8	62	2	34	246	20
F-9	63.56	0.64	32.16	235	21
F-10	64	2	32	239	23
F-11	62.21	0.64	33.56	250	25
F-12	62	2	33	249	22.3
F-13	62	4	32	231	23
F-14	62	4	32	233	20

Table 3: Calibration Values

Concentration(µg/ml)	Absorbance's
0	0
2	0.194
4	0.369
6	0.556
8	0.725
10	0.893

Table 4: Solubility Study in different solvents

Solvent	Observed Solubility (µg/ml)
Methanol	48.08±0.32
Water	0.525±0.51
pH buffer 1.2	08.16±0.23
pH buffer 6.8	52.36±0.14
pH buffer 7.4	65.25±0.19

subsequently diluted using methanol until its final volume reached 1000 µg per ml. The drug's UV-Vis (UV 1700, Shimadzu, Japan) λmax was confirmed by scanning a sample solution containing this stock solution between 200 and 400 nm [12,13].

Five different concentrations of the standard solution from the standard stock solution: 2, 4, 6, 8, and 10 µg/ml. At 241 nm, with methanol serving as a blank, the absorbance of every solution was recorded. By plotting absorbance against each concentration, a calibration graph was created.

Analysis by using FTIR Spectrometer

A KBr disc was used for FTIR analysis on an FTIR spectrometer (8400S Shimadzu, Japan). Pellet was formed by pressing 10 mg of medication into a mixture of KBr and triturating it. The resulting spectra was acquired in the 4000-400 cm-1 wavelength band using Fourier transform infrared spectroscopy. After that, it was subjected to an infrared beam and a spectrum was measured digitally [14,15].

Differential Scanning Calorimetry (DSC)

A DSC thermogram of Azilsartan was taken using the Mettler DSC 1-star system, which is made in Switzerland by Mettler-Toledo. The medicine was sealed in a perforated aluminum pan and subjected to a constant heating rate of 10°C/min from 30 to 350°C while being purge-gassed in an amount of 20 ml/min with nitrogen [16,17].

Table 5a: Solubility of Azilsartan

Name of Excipients	Solubility (mg/ml)
Olive Oil	36.48±0.3
Eucalyptus Oil,	28.56±0.9
Almond Oil,	29.38±0.11
Tween 80	38.02±0.2
PEG-400	33.58±0.5

Table 5b**:** Formulation Table

Azilsartan	4ml (20mg dose)
Water	62%
Oilive Oil	2%
S_{mix}	33%

Solubility Determination

The solubility of a medicine below 10 mg/mL adversely impacts bioavailability owing to absorption issues, since solubility prediction is an important metric. The solubility of azilsartan was assessed in methanol and water at 37±0.5°C. In 10 mL of solution, the medication was put in an adequate quantity and then sealed with rubber stoppers in glass containers. The vials have been kept in an orbital shaking incubator at a temperature of 37±0.5°C for a whole day. Subsequent to agitation, the vials were incubated at 37±0.5°C for twelve hours in order to attain equilibrium [18–20]. The solution was subsequently passed through a 0.45 µm Millipore filter, and the resultant filtrate was evaluated using UV spectrophotometry at λmax 241 nm, with solubility assessed using the appropriate calibration curve.

Drug-Excipients Compatibility Study

FTIR and DSC study of azilsartan were done to understand the physical and chemical compatibilities [21].

Test of Solubility for Screening Oils, Surfactants, as well as Co-Surfactants

The solubility of Azilsartan was used to assess oils, surfactants, and co-surfactants. The solubility of Azilsartan was assessed in many oils (almond oil, eucalyptus oil, olive oil), surfactants (Tween 80, Span 20, Tween 20) [22], co-surfactants (polyethylene glycol (PEG-400), ethanol, propylene glycol). Azilsartan was administered in excess inside centrifuge tubes, to which 5 ml of each oil, surfactant, and co-surfactant was included. The mixtures were agitated in a vortex mixer for 15 minutes. Samples were centrifuged

Table 6: Findings of Assessment of Variation for Determined Response

Parameters	Values	
	Particle-Size	ZP
Model	Quadratic Model(Significant)	Quadratic Model(Significant)
Model p value	0.454	0.4238
Standard-Deviation	3.86	3.57
Mean	47.64%	22.93%
CV	05.40%	15.57%
R^2	0.1672	0.4104
Adequate Precision	1.5443	3.2601
Regression Equation	Y1 = 255.77 X1 + 243.30 X2 + 242.26 X3 + 0.5898 X1 X2 – 26.88 X1 X3 + 34.27 X2 X3	Y1 = 26.66 X1 + 19.06 X2 + 23.62 X3 – 4.38 X1 X2 – 1387 X1 X3 + 16.41 X2 X3

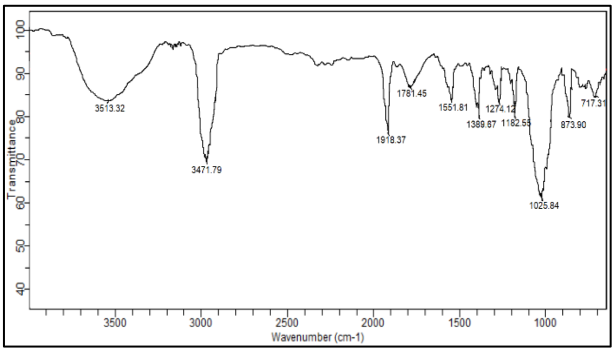

Figure 7 (A): FTIR spectra of Tween 80

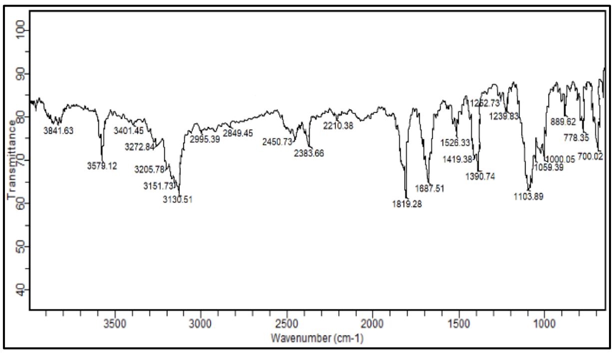

Figure 7 (B): FTIR spectra of Azilsartan and Physical Mixture

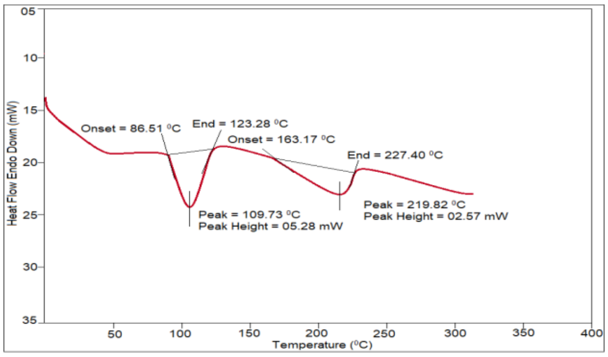

Figure 8: DSC spectra of Pectin

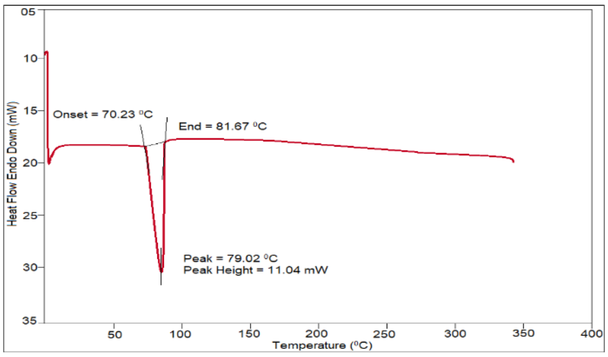

Figure 9: DSC-spectra of PEG-400

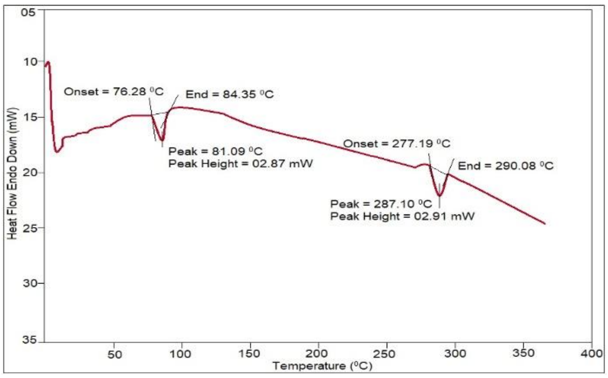

Figure 10: DSC-spectra of Tween 80

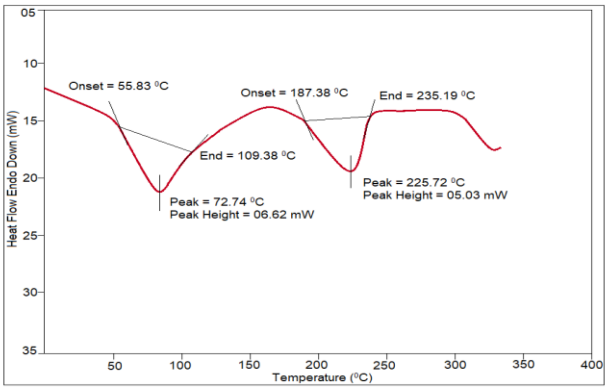

Figure 11: DSC-spectra of Azilsartan and Physical Mixture

for 15 minutes at 3000 rpm after 24 hours. The supernatant was then filtered using Whatman filter paper, diluted with methanol, and evaluated using UV-visible spectrophotometry at 241 nm, with all samples being repeated three times [21,23].

Preparation of Azilsartan loaded nano-emulsion

Using Zeta Potential (ZP) and transparency measurements, 14 designs were visually selected (Table 1) for Azilsartan loaded nano-emulsion [8,24]. For the desired volume of Nano-emulsion, the requisite quantity of Azilsartan was soluble in

Table 7: Characterization of Nano-emulsion

Formulation -code	pH-value of NE	Viscosity(cps)
F-1	6.92±0.02	1431±2.33
F-2	6.54±0.03	1530±2.28
F-3	6.55±0.04	1675±2.21
F-4	6.98±0.01	1721±2.29
F-5	6.12±0.03	1681±2.31
F-6	6.93±0.02	1525±2.35
F-7	6.45±0.01	1635±2.36
F-8	6.57±0.04	1751±2.39
F-9	6.58±0.06	1809±2.33
F-10	6.93±0.03	1745±2.27
F-11	6.56±0.02	1625±2.26
F-12	6.48±0.04	1865±2.21
F-13	6.29±0.05	1715±2.28
F-14	6.69±0.03	1678±2.31

Table 8: Characterization of Nano-emulsion

Code of Formulation	Drug Content (%)
F-1	86.19±0.27%
F-2	87.91±0.29%
F-3	88.92±0.30%
F-4	87.38±0.31%
F-5	96.91±0.32%
F-6	91.28±0.26%
F-7	92.38±0.28%
F-8	93.47±0.32%
F-9	94.21±0.31%
F-10	95.38±0.30%
F-11	91.36±0.28%
F-12	98.38±0.29%
F-13	90.65±0.35%
F-14	91.35±0.39%

Table 9: *In vitro* release pattern of Nano-emulsion

Time (Min.)	Pure Drug Solution	Nano-emulsion (F12)
0	0	0
60	17.42±1.36	15.21±1.59
120	51.04±2.41	21.02±2.18
180	70.62±1.69	36.63±1.91
240	90.52±2.32	51.52±2.21
300	-	71.10±2.61
360	-	85.25±2.60
420	-	90.01±2.55
480	-	98.25±2.59

the premeasured phase of oil. The necessary quantity of Smix was incorporated and thoroughly stirred in the beaker manually using an electromagnetic stirrer at ambient temperature. Then clear and transparent water that was distilled two times was dropped in until the solution became transparent. The prepared nano-emulsion was put into an appropriate container that was tightly sealed and stored in the room temperature [8,24].

Factorial Design

We have applied Simple Latex Design (SLD) in factorial design to optimize the formulation. The detailed methods are given below.

Optimization of Formula using SLD

Tables 1 and 2 show the OO:Smix (TW80:TP) ratios that were produced by blending TW80:TP (Smix) in different quantities with OO. Design-Expert (Stat-Ease Inc., USA) was used to monitor particle size (Y1) and ZP (Y2) as reactions to the water (X1), OO (X2), and Smix(X3) factors in improvement of N-NEs. Along with the statistical correlations between the independent variables, we also generated a 3D response surface map [25,26].

Characterization of Nano-emulsion

The optimized batch of formulated nano-emulsion was characterized by different instrumental techniques, all of which are discussed below:

Physical Evaluation

The color, transparency, homogeneity, and consistency of the produced nano-emulsion formulations were examined visually [11].

Droplet Dimensions and Size-Distribution

Using a Zetasizer(1000 HS, Malvern Instruments, Italy), the dimension of droplets was determined via photon correlation spectroscopy (PCS), that examines changes in light scattering induced by the Brownian motion of the droplets. A volumetric flask was used to combine 50 ml of water with 0.1 ml of nano-emulsion. The mixture was thoroughly shaken to mix, and light scattering at a 90° angle was measured at 25°C.

Analysis by ZP

A droplet's specific potential (ZP) is the sum of all the energies that the particle has absorbed in a given liquid. In order to determine how stable the mixture is while stored, it is essential to understand the nano-emulsion's ZP [27].

Assessment of pH

The pH parameters of the nano-emulsion have been determined at 25ºC employing a digital pH tester (pH system 362, Systronics, India). A 10% w/w dispersion was prepared by dispersing 1 g of nano-emulsion in 10 mL of distilled water. The initial calibration of the pH analyzer was performed with standard pH solutions (pH 4 and pH 7) [28]. The formulated products were subjected to pH analysis.

Investigation of Surface Morphology

We used scanning electron microscopy (SEM; SU3800/SU3900; Hitachi India) and transmission electron microscopy (TEM; HF5000; Hitachi India) to study the nano-emulsion's surface structure. After coating copper grids with Formvars, a nano-emulsion was applied and left to settle. The filtration paper was used to remove the extra liquid, which was then allowed to evaporate at room temperature for around 30 min. By using SEM/TEM, the dried grid that contained the nanoe-mulsion was examined.

In-vitro Release Study of Azilsartan Loaded Nano-emulsion Formulations

In vitro penetration investigations have been conducted utilizing a Franz diffusion cell (EDC-07; Electrolab India Pvt. Ltd.), a dependable technique for forecasting drug transport through the skin. The investigations were done using dialysis membranes. Receptor section of the diffusion cell had been stuffed with 25 ml of 7.4 pH phosphate buffer.

During the experiments, the whole setup was fastened to a magnetic stirrer and heated to 37±0.50°C. Magnetic beads were used to continuously mix the solution in the receptor compartment at 100 rpm [29].

Accelerated Stress Stability Study

The stability tests have been carried out for three months following the guidelines set forth by the ICH. After being optimized, the nano-emulsion batch was placed in an amber glass container and subjected to an accelerated stability chamber (CHM-10S, Remi Instruments Ltd., Mumbai, India) maintained at a temperature of 40°C±5°C and a relative humidity of 70%¹5%. The nano-emulsion was tested for size, pH, and polydispersity index (PDI) after three months [30,31].

RESULT AND DISCUSSION

The work seeks to develop and enhance a nano-emulsion including azilsartan to augment the solubility and bioavailability of the hypertension medication, which exhibits poor aqueous solubility. The results of the study are discussed below:

Physical Characterization and Identification

Physical examination and identification of the drug sample were conducted by the use of UV, FTIR, DSC, and solubility tests.

Physical examination

The substance appears as a white to off-white crystalline powder, with a color ranging from white to slightly yellowish. It has a distinctly bitter taste.

UV-Visible spectroscopy

Azilsartan was determined to have a peak absorption wavelength (λmax) of 241nm. Azilsartan exhibits two UV absorption peaks at 215 nm and 241 nm due to the presence of multiple conjugated systems within its structure (Figure 1). The peak at 215 nm corresponds to $\pi \rightarrow \pi^*$ electronic transitions in the biphenyl-tetrazole moiety, while the peak at 241 nm is attributed to $n \rightarrow \pi^*$ transitions in the oxadiazole ring. Additionally, intramolecular charge transfer (ICT) and possible tautomeric equilibria contribute to these distinct absorption maxima. The solvent polarity and pH may also influence the absorption behavior, leading to these two characteristic peaks.

Calibration Curve

The linearity of the calibration curve was seen in the 2-10µg/ml range, as shown in Table 3 and Figure 2, and the regression equation is $y = 0.0874x + 0.0113$. A score of 0.9993 indicates a correlation.

FTIR Analysis

In Figure 3, the subsequent groups are displayed by the FTIR spectra of Azilsartan: The ranges observed for N-H stretching are 3448.01 cm−1, C-H stretching is 3080.54 cm−1, C=O stretching is 1790.03 cm−1, C=O stretching is 1700.32 cm−1, C=O stretching is 1659.560 cm−1, C=C stretching is 1564.19 cm−1, and C-O stretching is 1129.86 cm−1.

DSC

Figure 4 shows the results of a DSC study of Azilsartan conducted at an examining assess of 10°C/min, which clearly showed a boiling an endothermic maximum at 191.38ºC. This peak shows the transition from a semisolid to a liquid form, which is associated with the transfer of heat. Moreover, the drug's molecules may degrade at higher temperatures, releasing energy as a byproduct. There should be a little exothermic peak at 239.73 ºC. The decomposition is shown by an endothermic peak on the DSC curve, which represents the heat absorbed during this specific thermal incident.

Solubility Determination

Mean-Particle-Size(Y1) ZP (Y2)

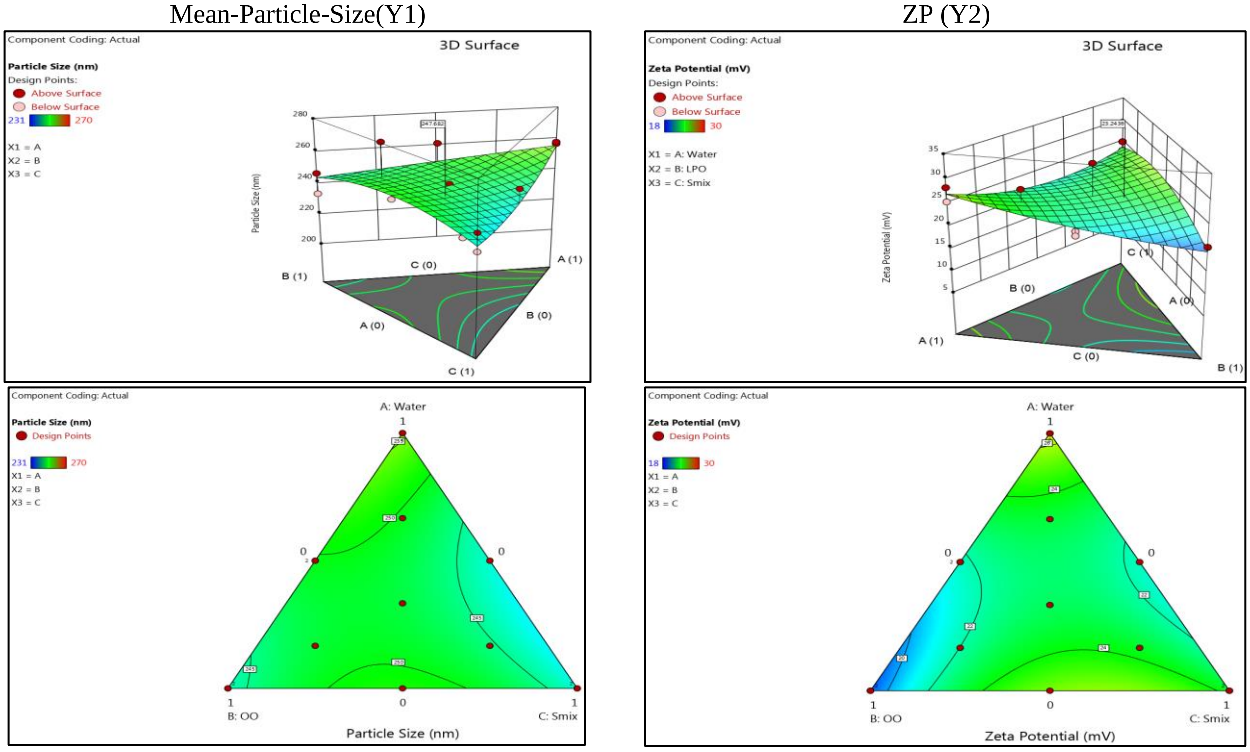

Figure 12: Response surface and Contour plots

The solubility of azilsartan was evaluated in several solvents, including methanol, water, and several buffers, at 37±0.5°C. Table 4 illustrates the solubility.

Drug-Excipients Compatibility Study

We have studied the drug-excipients compatibility studies by FTIR and DSC.

FTIR Analysis

Figures 5, 6, 7 (A) and 7 (B) illustrate the fractional Fourier transform infrared spectra of azilsartan and the physical mixture of all additives (pectin, Tween-80, and PEG-400). The spectral peaks of azilsartan were detected at the following wavelengths: 3448.01 cm−1 (N-H stretching); 3080.54 cm−1 (C-H stretching); 1790.03 cm−1 (C=O stretching); 1700.32 cm−1 (C=O stretching); 1659.56 cm−1 (C=O stretching); and 1564.19 cm−1 (C=C stretching). No substantial alterations were seen in the bands of the FTIR spectra for the optimized AZL-NE and pure azilsartan formulations, indicating an absence of molecular interaction between the medication and the formulation components. The spectra of the pure drug and AZL-NE preparation were consistent with the specified drug structure, confirming its compatibility with another additive.

DSC

It has been suggested that DSC may serve as a preliminary evaluation tool for assessing chemical and physical interactions among the constituents of a mixture. To find compatible excipients, the method compares the thermal profiles of pure compounds with a 1:1 physical mix of ingredients. The analysis of DSC thermogram showed that the drug has a melting point of 191.38 ºC. Pectin and Tween 80, when analyzed separately, had melting peaks of 86.51 ºC and 76.28 ºC, respectively, and when they were combined with azilsartan, these peaks did not show any

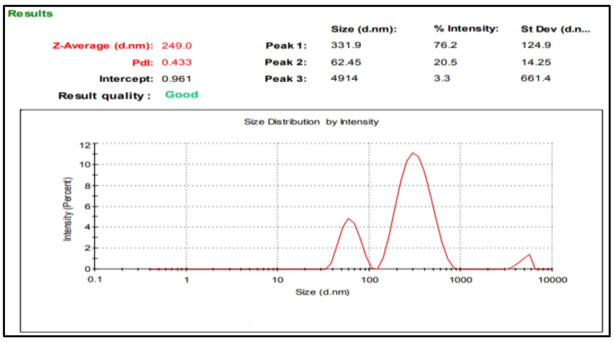

Figure 13: Particle size of F12 formulation

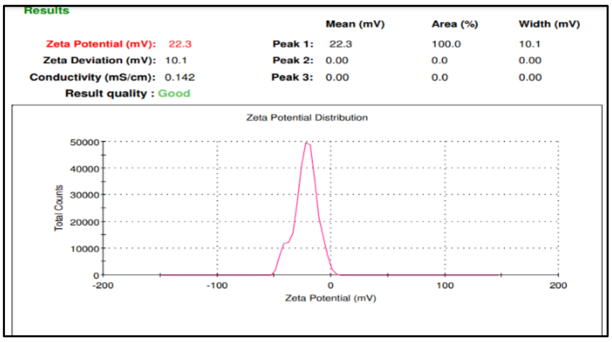

Figure 14: ZP of F12 formulation

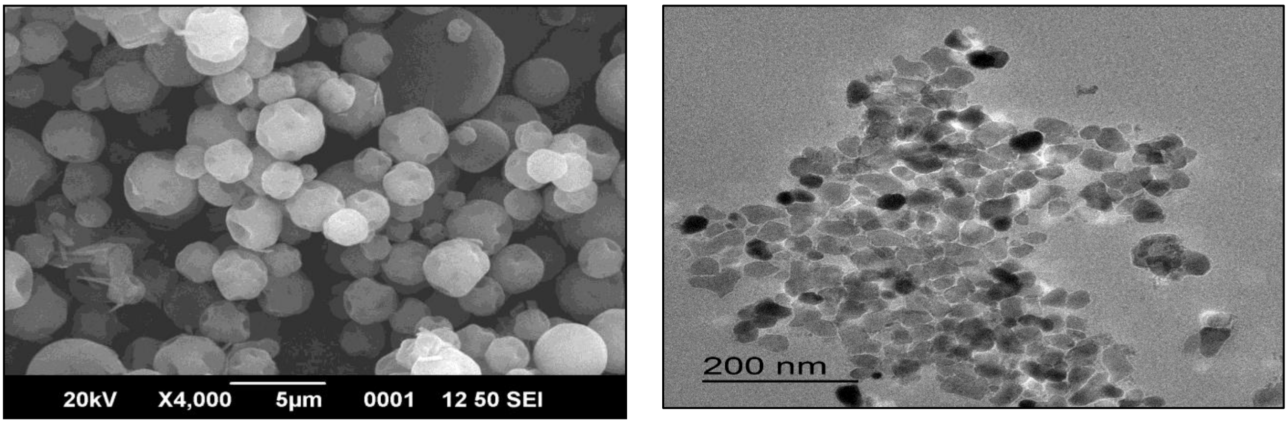

Figure 15: SEM (left) with TEM(right) image of F12 formulation

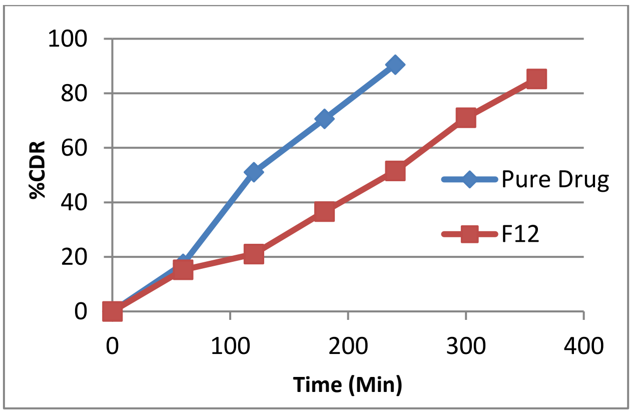

Figure 16: *In-vitro* drug release study of F12

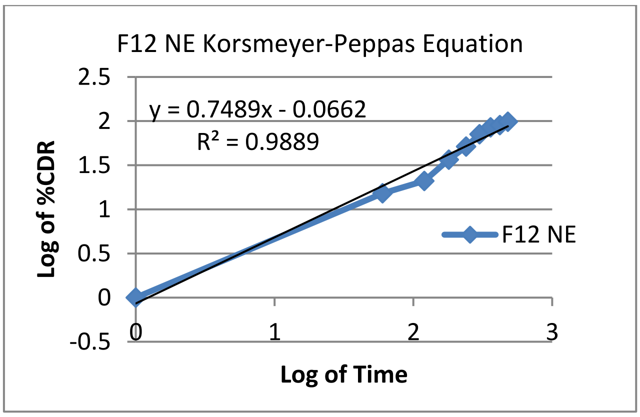

Figure 17: *In vitro* drug release study kinetics of F12

further changes. The absence of a change suggests that the drug together with pectin and Tween 80 are compatible. The DSC thermograms comparing azilsartan and individual excipients as well as drug-excipient combinations are presented in Figures 8-11.

Solubility Study for Oil, Surfactant, and Co-Surfactant Screening

The solubility profile for azilsartan is shown in Table 5A, and this information is used to screen oils, surfactants, and co-surfactants. The oil of choice was OO, with PEG-400 serving as a co-surfactant and Tween 80 as a surfactant.

Methods for developing and selecting Nano-emulsion

According to the factorial design (Table 5B), 14 formulations were chosen for the preparation of the Azilsartan loaded nano-emulsion based on visual observations such as transparency and pH.

The formulation of nano-emulsion F12 (composed of Drug 4 ml (20 mg) 62% water, 2% olive oil, and 33% Smix) involves a high-energy emulsification method. First, the oil phase (olive oil) and water phase (water) are prepared separately, ensuring that the oil is thoroughly mixed with the Smix (a blend of surfactants and co-surfactants). The oil phase is slowly added to the water phase with continuous stirring. After the initial mixing, the emulsion is subjected to high-shear homogenization using a high-pressure homogenizer or ultrasonicator to reduce the droplet size to the nano range (typically around 249 nm in this case). The process is repeated until the desired particle size and uniformity are achieved. The final nano-emulsion is then cooled and filtered to remove any large particles, ensuring stability and homogeneity.

Formulation Design

Table 6 shows the two components with coded and uncoded values for the bottom, middle, and higher design points. Responses from Y1 ranged from 18-30 mV and those from Y2 from 231-270 d. nm. Using the Design Expert® program, we fitted all of the observed responses from nine different formulations to a main effect model, which turned out to be the best match for Y1 and Y2. Tables 6 include the regression equations for each answer, as well as the values of R2 SD and % CV. The predicted outcome was substantial across all response variables, as shown by the ANOVA results in Tables 6, which include the dependent variables. The ZP was shown to be positively affected by independent variables X-1, X-2, and X-3, leading to the achievement of required particle size of the nano-formulation, also known as the nano-emulsion.

Model Assessment

Fit summaries were applied to the data in the Main Effect Model that the software had proposed after entering the data into the Design Expert® program for all the answers. ANNOVA was used to do the statistical assessment. The finding results may be seen in Tables 6. Coefficients that have many factors in the regression equation indicate variables that interact with one another. Furthermore, it demonstrates that variables and responses do not always follow a linear relationship. A factor's ability to elicit varying degrees of reactions depends on the number of factors being changed at once and their arrangement within the formulation.

Response Surface Plot Analysis

A low index of polydispersity signifies excellent homogeneity within the component populace, while a high polydispersity ratio implies significant size variation or many populations. The formulation batch producing the lowest particle size, as shown by the 3D reactive surfaces graph and the nanoparticulated structures, will be prioritized and identified as the optimized batch among all design batches. The F12 Design Batch, including a Smix concentration of around 33% and an olive oil content of 2%, demonstrates an ideal particle size of 249 nm.

ZP is crucial in finding the stability of nano-emulsions, making it a critical parameter in their formulation and characterization. In the field of nano-emulsions, ZP denotes the electric charge present on the surface of the scattered droplets within the system. A high absolute value of ZP indicates strong electrostatic repulsion between adjacent droplets, preventing their coalescence and aggregation. This electrostatic repulsion is crucial for maintaining the long-term stability of nano-emulsions by hindering the formation of larger droplets or phase separation. An optimal ZP, typically in the range of ±30 mV, ensures that the repulsive forces overcome attractive forces, such as van der Waals and hydrophobic interactions, thereby preventing destabilization of the nano-emulsion over time. By controlling and optimizing the ZP, formulators can enhance the physical stability and shelf life of nano-emulsions, making them suitable for various applications ranging from pharmaceuticals to food and cosmetic industries. Understanding and manipulating ZP in nano-emulsions are thus essential for achieving stable and functional formulations with improved dispersion, bioavailability, and overall performance.

The 3D response surface graph and values of regression coefficients indicated that the ZP of the nano-emulsion rose with elevated concentrations of olive oil, with no interactions or nonlinearity seen. It further confirmed that olive oil exerted a more significant influence on ZP. The Mean Particle Size (Y1) and ZP (Y2) for X1, X2, and X3 are shown in the response surface and contour plots depicted in Figure 12.

Figure 12 illustrated Contour plots in QbD are graphical representations that depict the relationship between critical quality attributes (CQAs) and critical process parameters (CPPs). These plots are useful for visualizing the design space and understanding how changes in process parameters may affect product quality. Both indicate a flat contour and that the critical quality attribute is relatively insensitive to changes in the associated process parameters within that region. This suggests a robust design space where variations in those parameters are less likely to impact product quality. Both response show optimized result in design space.

Characterization of Nano-emulsion

The detailed characterization parameters of the nano-emulsion is given below:

Physical characterisation

Every mixture is transparent, translucent, and homogeneous, exhibiting no roughness or impediments, as well as possess appropriate viscosity.

Distribution and Size of Droplet

The dimensions of the PNs are crucial as they affect the rate and extent of drug release and absorption. Smaller particles are observed to have higher bioavailability due to greater interface area available for drug absorption. The average particle size along with the solvent's refractive index, measuring angle, distribution variance, and the polydispersity index all require consideration for determination. Polydispersity index signifies the degree of homogeneity surrounding a particle population: lower values correspond to more homogeneous populations, while higher values indicate a more heterogeneous population characterized by wide size dispersion. The improved formulation batch (F12) obtained a mean particle size of 249 nm znd a PDI value of 0.433 (Figure 13).

Analysis of ZP

The ZP for the drug-loaded nano-emulsion is observed at 22.3 mV (shown in Figure 14). A high ZP value is associated with the stabilization of the nano-emulsion.

Study of Surface Morphology

Surface morphology of the nano-emulsion has been studied by SEM or TEM, indicating that the droplets had flawless layer. Droplets have a spherical form and have a diameter of 200-nm (Figure-15).

pH Measurement

Table 7 shows that the chosen NE (F1-14) formulation has a pH range of 6.12 to 6.98. The pH of NE is within the range of human skin (5-7), hence, is non-irrating to skin. Therefore, the NE formulations synthesized can be applied on the skin.

Viscosity Measurement

The viscosity of the nano-emulsion can be evaluated using the Brookfield viscometer with varying spindle speeds. The viscosity explains the rheological behavior of every nano-emulsion formulation. All those formulations showed a shear-thinning feature, which is when increased shear stress leads to decreased viscosity. Among all formulations, F12 exhibited the highest viscosity.

Drug Content of Nano-emulsion

The drug concentration in the nano-emulsion is anticipated to be reduced to some amount due to pectin, that consumes space as it expands in composition; this was quantified using a UV spectrometer at 241 nm. The percentage medication content of the nano-emulsion ranged from 82.77% to 98.38%, as shown in Table 8. The drug content % of formulations was within an acceptable limit.

In-vitro Release Study

The azilsartan-loaded emulsion from all batches was evaluated in vitro for the release of medication employing a Franz diffusion cell. In batch F12, the highest drug release was about 98.25±2.59%, as seen in Table 9. We tested the emulsion's in vitro release in PBS with a pH of 7.4 and a temperature of 37ºC. Figures 16 and 17 show the results of the nano-emulsion's 60-minute subjected to dialysis. Utilizing a UV-visible spectrophotometer, the amount of medication released was quantified by analyzing absorbance. There was an initial burst release of the medicine, that might have been caused by the smaller particles increasing the emulsion's surface area or by the medication diffusing through the emulsion's outer body.

Accelerated Stability Testing

The enhanced emulsion was subjected to stability tests. The results show that the Azilsartan-loaded nano-emulsion (F12 formulation batch) is stable under the given temperature and humidity conditions. The improved formulation (F12) stability study that was performed over three months, reviewing certain features at regular intervals. The pH showed a gradual decrease over time, moving from 6.48 ± 0.03 to 6.33 ± 0.03 after three months. Viscosity reduced slightly during this time from 1865 ± 2.21 cps to 1851 ± 2.30 cps by the end of the study period. The drug content was stable throughout this period as well, starting at 98.38 ± 0.29% and ending at 98.29 ± 0.39% after three months. The stability testing of the formulation indicated that it preserved its core properties with little modifications. The FTIR analysis of Azilsartan revealed characteristic bands corresponding to various functional groups, including N-H stretching, C-H stretching, and C=O stretching. The DSC study revealed a clear boiling endothermic maximum at 191.38ºC, indicating the conversion between a partially solid to a liquid condition. Furthermore, a small exothermic peak at 239.73 ºC indicated decomposition. The compatibility analysis with excipients using FTIR and DSC validated the lack of substantial molecular interactions. For the nano-emulsion formulation, a factorial design approach was employed, and 14 formulations were selected based on visual observations and pH. The results of the ANOVA for particle size and ZP demonstrated that factors like olive oil concentration (X1), Tween 80 concentration (X2), and PEG-400 concentration (X3) had a significant impact on achieving the desired properties. Response surface and contour plots further illustrated the relationship between critical parameters and quality attributes, indicating a robust design space. The physical evaluation of the nano-emulsion revealed clean, transparent, and homogeneous formulations with appropriate consistency. The improved formulation (F12) demonstrated a mean particle size of 249 nm and a ZP of -22.3 mV, indicating stability. The SEM and TEM pictures exhibited smooth-surfaced, spherical droplets measuring 200 nm in diameter. The pH readings were within the dermally compatible range (5-7). Viscosity assessments indicated shear-thinning characteristics, with F12 exhibiting greater viscosity. The drug concentration varied between 82.77% and 98.38%, remaining within safe parameters. In the in-vitro release investigation, F12 exhibited a prolonged drug release profile across time. Accelerated stability experiments validated the stability of F12 over a three-month period, with negligible changes in pH, viscosity, and drug content. In conclusion, the formulated nano-emulsion (F12) of Azilsartan demonstrated advantageous physicochemical characteristics, prolonged drug release, and stability, positioning it as a viable option for further pharmaceutical development and possible therapeutic uses.

CONCLUSION

In conclusion, this study successfully developed and characterized an optimized nano-emulsion formulation for azilsartan, addressing its poor aqueous solubility and potentially enhancing its bioavailability. The systematic approach using factorial design and comprehensive characterization techniques led to a stable formulation with desirable physicochemical properties. Key findings include: Successful formulation of azilsartan-loaded nano-emulsion with optimal particle size (249 nm) and ZP (-22.3 mV). Compatibility between azilsartan and selected excipients confirmed through FTIR and DSC analyses. Robust design space identified through response surface methodology and contour plots. Sustained drug release profile observed in in-vitro studies, with 98.25% release over 8 hours; and stability of the optimized formulation (F12) confirmed under accelerated conditions for 3 months. These results suggest that the developed nano-emulsion formulation could potentially improve the therapeutic efficacy of azilsartan by enhancing its solubility and bioavailability. Further in-vivo studies are warranted to confirm the pharmacokinetic advantages and clinical efficacy of this formulation.

REFERENCES

1. Ratria, A. K.; Muhammad, R. R.; Nurrobi Y. A. Right Ventricular Free-Wall Longitudinal Strain (RVfwLS) as a Prognostic Factor in Patients with Pulmonary Arterial Hypertension (PAH): A Systematic Review and Meta-Analysis. J. Med. Pharm. Chem. Res. 2025, 7 (December 2024), 2384–2396.
2. Arina, C. A.; Khairina, Y. Gender Differences in Risk Factors and Clinical Profiles of Ischemic Stroke Patients: A Cross-Sectional Study at Adam Malik Hospital, Medan. J. Med. Pharm. Chem. Res. 2025, 7 (7), 1496–1502. https://doi.org/10.48309/jmpcr.2025.479250.1438.
3. Ito, S.; Nishiyama, Y.; Sugiura, K.; Enya, K. Safety and Efficacy of Azilsartan in Paediatric Patients with Hypertension: A Phase 3, Single-Arm, Open-Label, Prospective Study. Clin. Exp. Nephrol. 2022, 26 (4), 350–358. https://doi.org/10.1007/s10157-021-02159-9.
4. Mizutani, N. Effect of Azilsartan on Blood Pressure in Patients with Uncontrolled Hypertension. Ther. Res. 2013, 34 (4), 507–519.
5. Pradhan, A.; Tiwari, A.; Sethi, R. Azilsartan: Current Evidence and Perspectives in Management of Hypertension. Int. J. Hypertens. 2019. https://doi.org/10.1155/2019/1824621.
6. Shakeel, F.; Faisal, M. S. Nanoemulsion: A Promising Tool for Solubility and Dissolution Enhancement of Celecoxib. Pharm. Dev. Technol. 2010, 15 (1), 53–56. https://doi.org/10.3109/10837450902967954.
7. Shaikh, N. M.; Vijayendra Swamy, S. M.; NARSING, N. S.; Kulkarni, K. B. Formulation and Evaluation of Nanoemulsion for Topical Application. J. Drug Deliv. Ther. 2019, 9 (4-s), 370–375. https://doi.org/10.22270/jddt.v9i4-s.3301.
8. Kumar, G.; Virmani, T.; Pathak, K.; Kamaly, O. Al; Saleh, A. Central Composite Design Implemented Azilsartan Medoxomil Loaded Nanoemulsion to Improve Its Aqueous Solubility and Intestinal Permeability: In Vitro and Ex Vivo Evaluation. Pharmaceuticals 2022, 15 (11). https://doi.org/10.3390/ph15111343.
9. Kumar, R. R.; Krishnan, K.; Sankararao, G.; Samathoti, P.; Kumar, J. P.; Bagade, O. M.; Teja, K.; Reddy, K.; Gopisetti, J. Biochemical Engineering of Green Nanomaterials for Targeted Drug Delivery and Therapeutic Applications. J. Chem. Rev. 2025, 6 (3), 191–215.
10. Vattikundala, P.; Chaudhary, S.; Sumithra, M. Design, Preparation, Characterization, and Evaluation of NR4A1 Agonist Novel 6-Mercaptopurine Monohydrate Loaded Nanostructured Lipid Carriers Suspension for Enhanced Solubility and Invivo Bioavailability. J. Med. Pharm. Chem. Res. 2025, 7 (6), 1059–1078. https://doi.org/10.48309/jmpcr.2025.472478.1381.
11. Rajab, N. A.; Jassem, N. A. A Design and in Vitro Evaluation of Azilsartan Medoxomil as a Self-Dispersible Dry Nanosuspension. Der Pharm. Sin. 2018, 9 (1), 12–32.
12. Adeeyinwo, C. E.; Okorie; Idowu, G. O. Basic Calibration of UV/ Visible Spectrophotometer. Int. J. Sci. Technol. 2013, 2 (3), 247–251.
13. Singh, R.; Wu, B.; Tang, L.; Liu, Z.; Hu, M. Identification of the Position of Mono- O-Glucuronide of Flavones and Flavonols by Analyzing Shift in Online UV Spectrum (Λmax) Generated from an Online Diode Array Detector. J. Agric. Food Chem. 2010, 58 (17), 9384–9395. https://doi.org/10.1021/jf904561e.
14. Fadlelmoula, A.; Pinho, D.; Carvalho, V. H.; Catarino, S. O.; Minas, G. Fourier Transform Infrared (FTIR) Spectroscopy to Analyse Human Blood over the Last 20 Years: A Review towards Lab-on-a-Chip Devices. Micromachines 2022, 13 (2). https://doi.org/10.3390/mi13020187.
15. Munajad, A.; Subroto, C.; Suwarno. Fourier Transform Infrared (FTIR) Spectroscopy Analysis of Transformer Paper in Mineral Oil-Paper Composite Insulation under Accelerated Thermal Aging. Energies 2018, 11 (2). https://doi.org/10.3390/en11020364.
16. Mutlur, S. Thermal Analysis of Composites Using DSC. Adv. Top. Charact. Compos. 2004, 11–33.
17. Schick, C. Differential Scanning Calorimetry (DSC) of Semicrystalline Polymers. Anal. Bioanal. Chem. 2009, 395 (6), 1589–1611. https://doi.org/10.1007/s00216-009-3169-y.
18. Ghanbari, A.; Sarbaz, Y.; Jouyban-Gharamaleki, V.; Jouyban-Gharamaleki, K.; Soleymani, J.; Jouyban, A. An Improved Automated Setup for Solubility Determination of Drugs. Pharm. Sci. 2016, 22 (3), 210–214. https://doi.org/10.15171/PS.2016.33.
19. Xu, H.; Kang, L.; Qin, J.; Lin, J.; Xue, M.; Meng, Z. Solubility of Azilsartan in Methanol, Ethanol, Acetonitrile, n-Propanol, Isopropanol, Tetrahydrofuran, and Binary Solvent Mixtures between 293.15 and 333.15 K. ACS Omega 2020, 5 (11), 6141–6145. https://doi.org/10.1021/acsomega.0c00156.
20. Gao, L.; Zhang, X. R. Synthesis of Two Novel Azilsartan Cocrystals: Preparation, Physicochemical Characterization and Solubility Studies. Crystals 2020, 10 (9), 1–11. https://doi.org/10.3390/cryst10090739.
21. Doğantürk, M.; Seçilmiş Canbay, H. Drug-Excipient Compatibility Studies In Binary Mixtures of Tadalafil

by Using DSC, TGA and FTIR. Süleyman Demirel Üniversitesi Sağlık Bilim. Derg. 2023, 14 (2), 130–141. https://doi.org/10.22312/sdusbed.1197638.
22. Atun, S.; Aznam, N.; Arianingrum, R.; Priastomo, Y.; Cholil, R. Nanoemulsion Formulation Gel and Ethanol Extract of Aloe Vera , Antibacterial Activity Test , and Prediction of Interaction Mechanism against PBP-3 Receptor. J. Med. Chem. Sci. 2025, 8, 106–118. https://doi.org/10.26655/JMCHEMSCI.2025.1.10.
23. Patel, P.; Ahir, K.; Patel, V.; Manani, L.; Patel, C. Drug-Excipient Compatibility Studies : First Step for Dosage Form Development. Pharma Innov. J. 2015, 4 (5), 14–20.
24. Jassem, N. A.; Rajab, N. A. Formulation and in Vitro Evaluation of Azilsartan Medoxomil Nanosuspension. Int. J. Pharm. Pharm. Sci. 2017, 9 (7), 110. https://doi.org/10.22159/ijpps.2017v9i7.18917.
25. Sharma, P.; Tailang, M. Design, Optimization, and Evaluation of Hydrogel of Primaquine Loaded Nanoemulsion for Malaria Therapy. Futur. J. Pharm. Sci. 2020, 6 (1). https://doi.org/10.1186/s43094-020-00035-z.
26. Mehmood, T.; Ahmad, A.; Ahmed, A.; Ahmed, Z. Optimization of Olive Oil Based O/W Nanoemulsions Prepared through Ultrasonic Homogenization: A Response Surface Methodology Approach. Food Chem. 2017, 229, 790–796. https://doi.org/10.1016/j.foodchem.2017.03.023.
27. Nanocomposix. Zeta Potential Analysis of Nanoparticles. Nanocomposix Publ. 2012, 1–6.
28. Takeshita, Y.; Johnson, K. S.; Coletti, L. J.; Jannasch, H. W.; Walz, P. M.; Warren, J. K. Assessment of PH Dependent Errors in Spectrophotometric PH Measurements of Seawater. Mar. Chem. 2020, 223. https://doi.org/10.1016/j.marchem.2020.103801.
29. Li, N.; Fan, L.; Wu, B.; Dai, G.; Jiang, C.; Guo, Y.; Wang, D. Preparation and in Vitro/in Vivo Evaluation of Azilsartan Osmotic Pump Tablets Based on the Preformulation Investigation. Drug Dev. Ind. Pharm. 2019, 45 (7), 1079–1088. https://doi.org/10.1080/03639045.2019.1593441.
30. Parveen, R.; Baboota, S.; Ali, J.; Ahuja, A.; Ahmad, S. Stability Studies of Silymarin Nanoemulsion Containing Tween 80 as a Surfactant. J. Pharm. Bioallied Sci. 2015, 7 (4), 321–324. https://doi.org/10.4103/0975-7406.168037.
31. Khan, N. U.; Ali, A.; Khan, H.; Khan, Z. U.; Ahmed, Z. Stability Studies and Characterization of Glutathione-Loaded Nanoemulsion. J. Cosmet. Sci. 2018, 69 (4), 257–267.