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ORIGINAL ARTICLE

In Vivo Pharmacokinetic Evaluation of Optimized Nanoemulsion of Azilsartan by HPLC

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ABSTRACT
This study aimed to evaluate the in vivo pharmacokinetics of Azilsartan using an optimized nanoemulsion formulation, compared to pure Azilsartan. A high-performance liquid chromatography (HPLC) method was developed for the quantification of Azilsartan, with optimization of the mobile phase, column, and other chromatographic conditions. The pharmacokinetic evaluation was performed in male Wistar rats after oral administration of either pure Azilsartan or the optimized Azilsartan-loaded nanoemulsion at a dose of 0.67 mg/kg. The optimized nanoemulsion exhibited significant improvements in drug absorption, as indicated by the higher Cmax (3678.80 ± 49.83 ng/mL) compared to the pure Azilsartan (2308.75 ± 90.99 ng/mL). The area under the curve (AUC_0-T) and the mean residence time (MRT) were also notably higher for the nanoemulsion, suggesting enhanced bioavailability and prolonged drug release. These findings indicate that the optimized nanoemulsion formulation improves the pharmacokinetic profile of Azilsartan, offering a promising approach for enhancing therapeutic efficacy.
Keywords*: Nanoemulsion; Azilsartan; HPLC; Pharmacokinetics; Method development*

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INTRODUCTION

Azilsartan, an angiotensin II receptor antagonist (ARB), is widely used for the treatment of hypertension and related cardiovascular diseases (1,2). Due to its high lipophilicity and low aqueous solubility, Azilsartan presents challenges in achieving optimal bioavailability. These pharmacokinetic limitations necessitate innovative drug delivery systems to enhance its solubility, stability, and absorption (3–5). Nanoemulsions, as nanoscale emulsions, have emerged as a promising solution for improving the pharmacokinetics of poorly water-soluble drugs like Azilsartan (6). By decreasing the particle size to the nanometer range, nanoemulsions improve the solubility of hydrophobic drugs, offering enhanced drug delivery, higher bioavailability, and more controlled therapeutic action (7–10).

The use of nanoemulsions in drug delivery systems is supported by their ability to improve drug dissolution rates and facilitate drug penetration across biological membranes (11–13). Nanoemulsions, typically composed of surfactants, co-surfactants, and oils, form stable, submicron-sized droplets that enhance drug absorption via the lymphatic system, bypassing first-pass metabolism in the liver (14,15). Furthermore, the formulation's optimization is key to maximizing its therapeutic potential, ensuring consistent release profiles, and minimizing adverse effects.

The primary goal of this study was to evaluate the pharmacokinetic profile of an optimized Azilsartan-loaded nanoemulsion, using high-performance liquid chromatography (HPLC) for quantification and analysis. HPLC is a robust and reliable technique for the separation and detection of drugs in biological samples due to its precision, sensitivity, and ability to handle complex matrices (16–18). Through the application of this technique, the absorption, distribution, metabolism, and elimination (ADME) of the optimized nanoemulsion formulation *in vivo* were systematically evaluated (19).

We already formulated and optimized the nanoemulsion of Azilsartan, taking into account critical parameters such as droplet size, zeta potential, drug loading capacity, and stability. The research paper containing formulation section is already published in International *Journal of Drug Delivery and*

Technology (20). Following formulation, the pharmacokinetic parameters were assessed through *in vivo* testing in animal models, with plasma concentration levels of Azilsartan quantified at predetermined time points. This evaluation was essential for understanding how the nanoemulsion formulation affects the systemic absorption and overall pharmacokinetic behavior of Azilsartan compared to conventional oral administration. Overall, the research aims to provide valuable insights into the enhancement of Azilsartan's pharmacokinetic profile through nanoemulsion-based drug delivery, thus contributing to improved therapeutic outcomes for patients requiring hypertension management. This study holds promise for the development of more effective and efficient drug formulations, potentially extending the scope of nanoemulsions in the clinical management of cardiovascular diseases.

MATERIAL AND METHODS

Chemicals

All the required chemical was purchased and procured from Lab Trading Laboratory, Aurangabad, Maharashtra, India.

Method Development for Azilsartan

Preparation of stock solution

The stock solution of Azilsartan was prepared by dissolving 5 mg of the drug in 10 mL of solvent, resulting in a concentration of 0.5 mg/mL, equivalent to 500,000 ng/mL. For the first dilution, 3.2 mL of the stock solution was diluted to 10 mL, yielding a concentration of 0.16 mg/mL, or 160,000 ng/mL. Subsequently, 1.0 mL of this first dilution was further diluted to 10 mL, resulting in a final concentration of 0.016 mg/mL, equivalent to 16,000 ng/mL (21).

Preparation of Buffer

6.30g of Ammonium formate was dissolved in 1 Lt of HPLC grade water, adjust its pH-3.0 with formic acid and filtered through 0.45µ membrane filter paper.

Preparation of Mobile Phase

An Acetonitrile was used in different combination with buffer, and TFA (Trifluoroacetic acid) were mixed in the different ratios such of 30:70, 60:40, 50:50, and 20:80 then filtered through 0.45µ membrane filter paper. The different mobile phases used for the trials along with observations are tabulated in **Table 1**.

Table 1: Trails during method development of Azilsartan

Trial No.	Mobile Phase	Column	Observation
Trial 1	Acetonitrile : 0.1% TFA (70:30)	Agilent Eclipse XDB (250x4.6mm, 5µ)	System suitability conditions are not within the limit.
Trial 2	Acetonitrile : 0.1% TFA (60:40)	Agilent Eclipse XDB (250x4.6mm, 5µ)	Retention time is not within the limit.
Trial 3	Acetonitrile : 0.1% TFA (50:50)	Waters X-Terra RP-18 (150x4.6mm, 3.5µ)	Baseline is not sufficient.
Trial 4	ACN: Ammonium formate pH-3.0/Formic acid (20:80)	Waters X-Terra RP-18 (150x4.6mm, 3.5µ)	Broad peaks are observed.
Trial 5	ACN : Ammonium formate pH-3.0/Formic acid (30:70)	Waters X-Terra RP-18 (150x4.6mm, 3.5µ)	This method is suitable for validation.

Calibration Curve of Azilsartan

The calibration curve of pure Azilsartan was constructed using optimized chromatographic conditions. A Waters Alliance HPLC quaternary pump equipped with a PDA detector was employed for the analysis. The separation was performed on a Waters X-Terra RP-18 column (150 mm x 4.6 mm, 3.5 µm particle size). The injection volume was set at 10 µL, and the column was maintained at ambient temperature. The mobile phase was delivered at a flow rate of 1 mL/min, with a total run time of 5.0 minutes.

The Concentration Vs Peak response data for Azilsartan showed a strong linear relationship, with increasing concentration (800–8000 ng/mL) corresponding to proportionally higher peak response. The concentrations and relevant peak area data is given in Table 2 and the calibration curve is depicted in Figure 1.

Table 2: Calibration results of Pure Azilsartan

S.no	Concentration (ng/mL)	Peak Response
1	800.00	10312
2	1600.00	20758
3	2400.00	31124
4	3200.00	41163
5	4000.00	51723
6	4800.00	62347
7	5600.00	72266
8	6400.00	82541
9	7200.00	93057
10	8000.00	103116

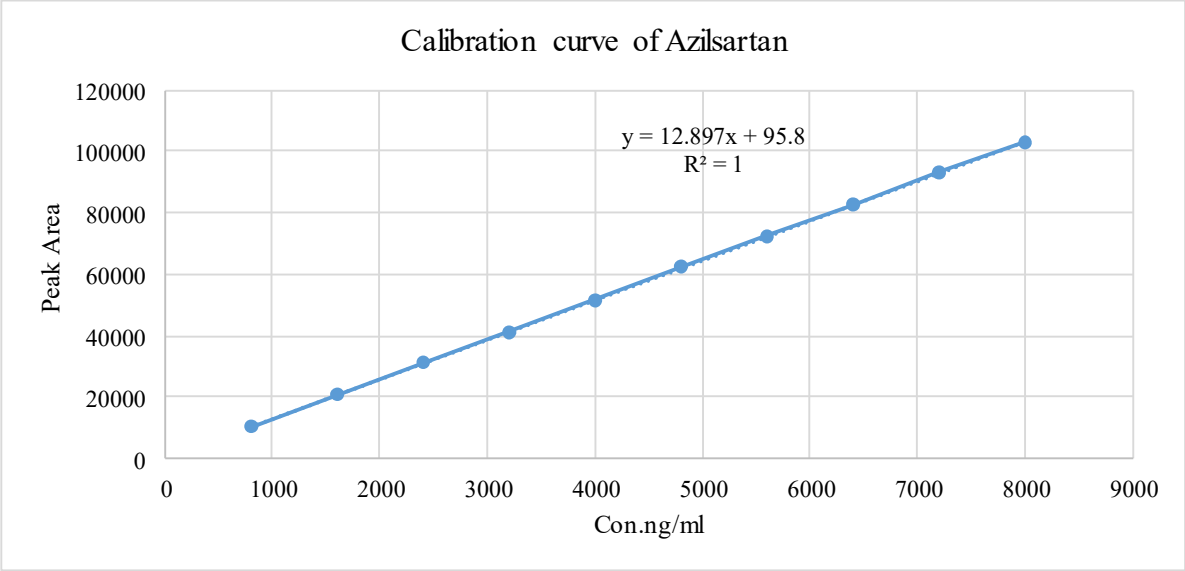


Figure 1: Calibration curve of Pure Azilsartan

***In vivo* Pharmacokinetic Studies of Developed Optimized Azilsartan-loaded Nanoemulsion**

Animals Used and Ethical Approval

The present study was conducted using Male Wistar rats, the protocol was approved by IAEC committee of Systemic Life Sciences and Research Pvt. Ltd. Telangana, India with ethical approval number 17/IAEC-II/SLSRPL/2024. The animals were divided into the two groups, Group-I received pure Azilsartan and group-II which received optimized nanoemulsion formulation. The dose of pure Azilsartan was administered as 0.67 mg/kg body weight of rat. The equivalent quantity of formulation was taken which contain 0.67 mg of Azilsartan i.e. 3.35 mL of nanoemulsion as formulation contains 20 mg of drug per 100 mL.

Procedure

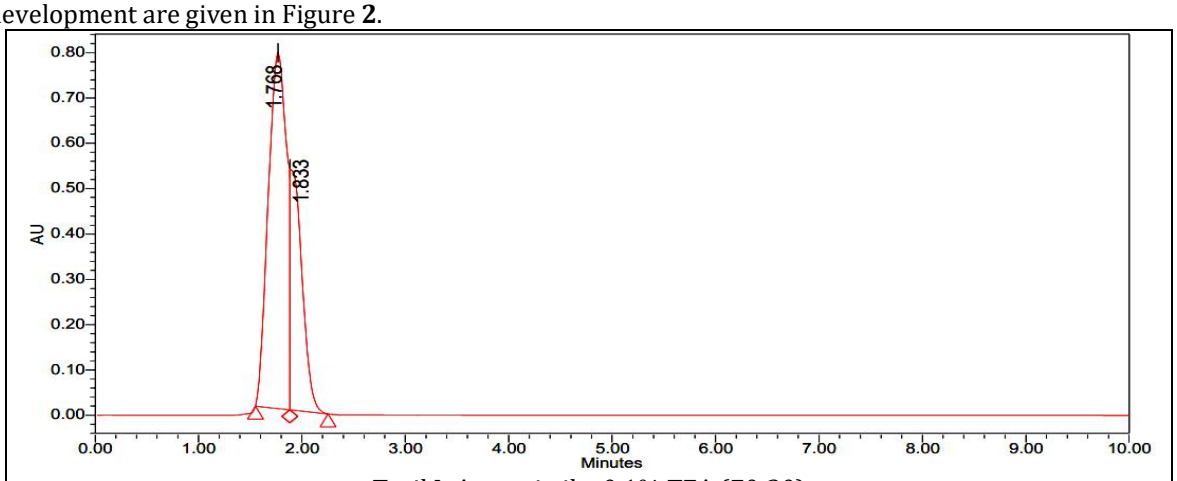
Azilsartan, in both its pure and optimized formulations, was administered orally to rats at a dose of 0.67 mg/kg body weight. A stock solution with an initial concentration of 4000 ng/mL was prepared using serial dilutions to ensure accurate quantification. Specifically, 5 mg of Azilsartan was dissolved in 10 mL, followed by further dilutions of 3.2 mL into 10 mL, 1 mL into 10 mL, and 0.5 mL into 2 mL to achieve the desired working concentrations. Blood samples were collected at predetermined intervals—0, 1, 2, 3, 4, 5, 7, 9, 11, and 13 hours post-dosing—to assess plasma drug concentrations. The collected blood samples were processed to isolate plasma, which was subsequently analyzed using a HPLC method.

RESULTS AND DISCUSSION

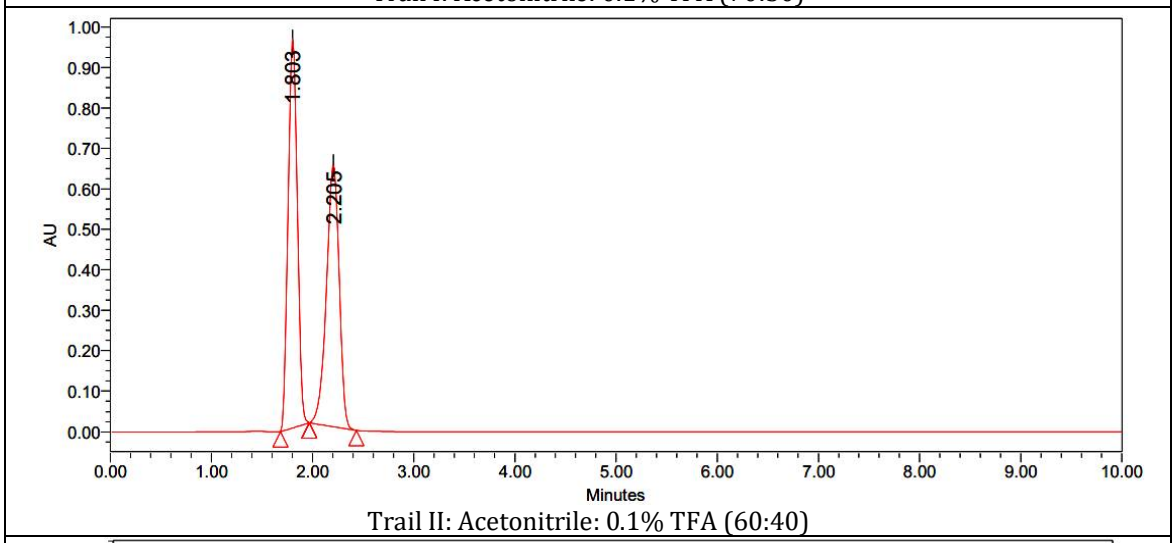
Method Development

To develop optimized chromatographic method to estimate Azilsartan quantitatively, different mobile phases were used and the most optimized chromatogram and well resolute peak was obtained from Trial 5. Where we used mobile phase as ACN: Ammonium formate pH-3.0/Formic acid (30:70) and Waters X-

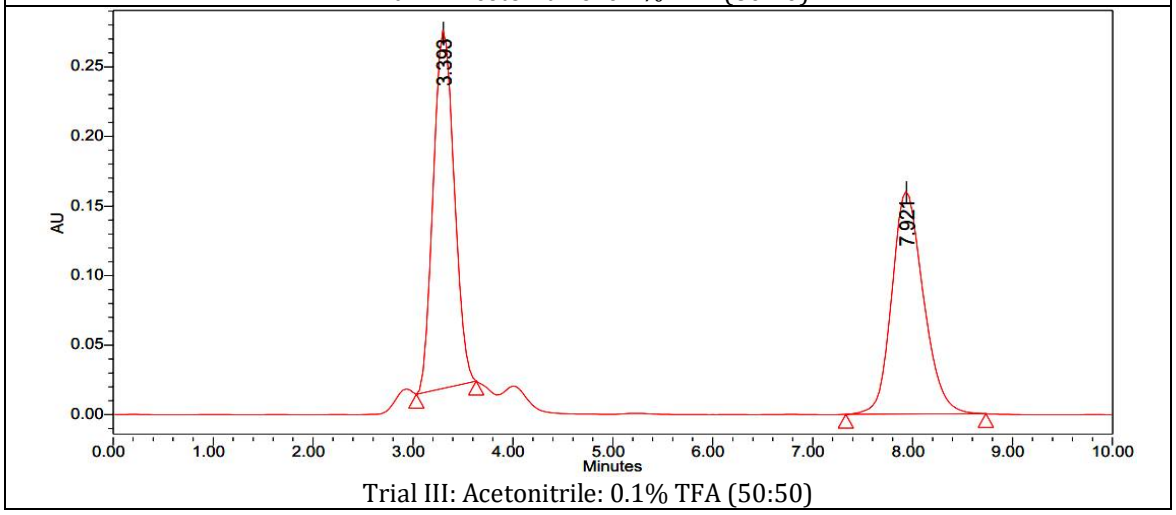
Terra RP-18 (150x4.6mm, 3.5µ) as HPLC column. The different chromatograms obtained during method development are given in Figure **2**.



Trail I: Acetonitrile: 0.1% TFA (70:30)



Trail II: Acetonitrile: 0.1% TFA (60:40)



Trial III: Acetonitrile: 0.1% TFA (50:50)

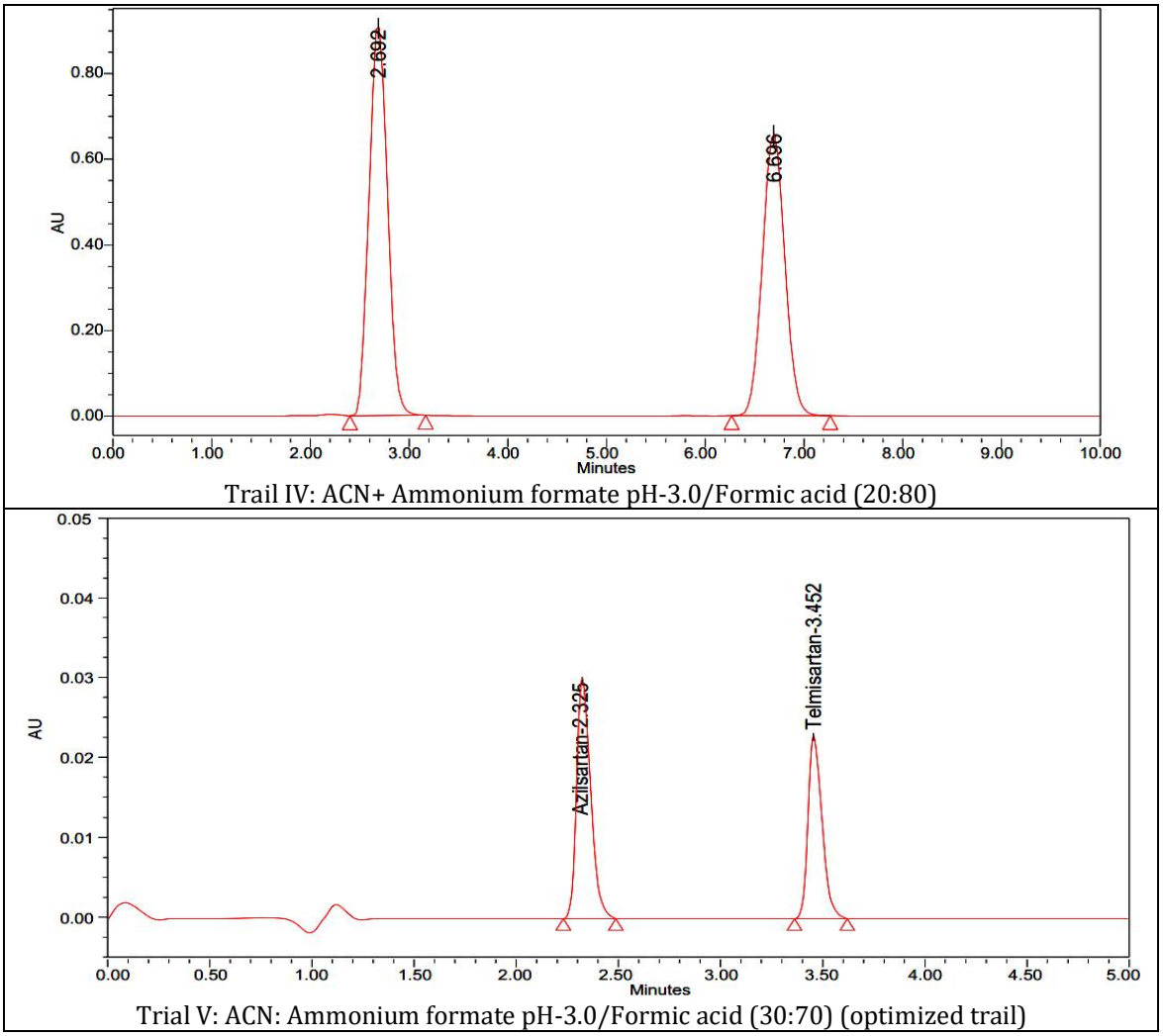


Figure 2: The trial and optimized chromatograms obtained from method development of pure Azilsartan

***In vivo* Pharmacokinetic Studies of Developed Optimized Azilsartan-loaded Nanoemulsion**

The results of *in vivo* plasma drug concentration in control and test animals are tabulated in **Table 3**. The pharmacokinetic evaluation of Control (Pure Azilsartan) and Test (Optimized nanoemulsion) reveals significant improvements in the drug's performance with the nanoemulsion formulation. Both formulations exhibited the same Tmax (3.00 ± 0.00 hrs), indicating that the time to reach peak plasma concentration was unaffected by the formulation. However, the Cmax for the nanoemulsion (3678.80 ± 49.83 ng/mL) was substantially higher than the pure form (2308.75 ± 90.99 ng/mL), demonstrating enhanced drug absorption. The T1/2 was slightly prolonged for the nanoemulsion (2.43 ± 0.02 hrs) compared to the pure form (2.03 ± 0.01 hrs), suggesting improved retention and slower elimination. The area under the curve values, AUC_0-T (22437.29 ± 43.32 ng *hrs/mL vs. 12776.31 ± 55.28 ng*hrs/mL) and AUC_0-∞ (24529.07 ± 49.83 ng*hrs/mL vs. 13620.49 ± 90.99 ng* hrs/mL), indicate a marked increase in the total drug exposure with the nanoemulsion (**Table 4**). Furthermore, the MRT was higher for the nanoemulsion (6.66 ± 0.12 hrs) than the pure form (5.90 ± 0.15 hrs), highlighting prolonged drug release and residence time. These findings demonstrate that the optimized nanoemulsion significantly enhances the bioavailability and pharmacokinetic profile of Azilsartan, making it a promising approach for improving therapeutic efficacy. The plasma time profile of Azilsartan and optimized nanoemulsion is given in **Figure 3**.

Table 3: In vivo plasma drug concentration in control and test animals

Time (hrs)	Control (Pure Azilsartan)	Test (Optimized nanoemulsion)
	Mean ± SD (ng/mL)	Mean ± SD (ng/mL)
0.0	0.0 ± 0.0	0.0 ± 0.0
1.0	476.31 ± 9.8	1517.74 ± 5.11
2.0	1250.69 ± 55.28	2764.41 ± 43.32
3.0	2308.75 ± 90.99	3678.8 ± 49.83
4.0	2056.92 ± 23.65	3126.23 ± 58.9
5.0	1667.73 ± 38.64	2622.79 ± 47.16
7.0	1130.18 ± 33.17	1868.42 ± 42.15
9.0	672.29 ± 6.10	1187.43 ± 41.84
11.0	288.31 ± 7.19	596.81 ± 10.46
13.0	0.0 ± 0.0	0.0 ± 0.0

The values are expressed as mean ± SD (n=6)

Table 4: Pharmacokinetics of pure Azilsartan and Optimized nanoemulsion

Parameter	Control (Pure Azilsartan)	Test (Optimized nanoemulsion)
Tmax (hrs)	3.00 ± 0.00	3.00 ± 0.00
Cmax (ng/mL)	2308.75 ± 90.99	3678.80 ± 49.83
T1/2 (hrs)	2.03 ± 0.01	2.43 ± 0.02
AUC_0-T (ng*hrs/mL)	12776.31 ± 55.28	22437.29 ± 43.32
AUC_0-∞ (ng*hrs/mL)	13620.49 ± 90.99	24529.07 ± 49.83
MRT (hrs)	5.90 ± 0.15	6.66 ± 0.12

The values are expressed as mean ± SD (n=6)

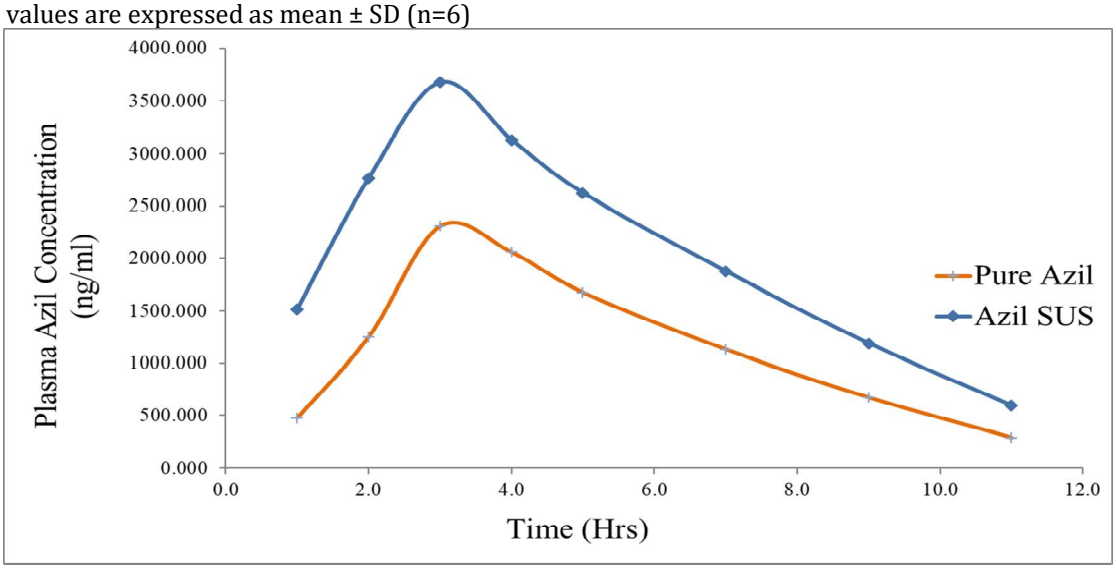


Figure 3: Plasma time profile of pure Azilsartan and Optimized Nanoemulsion

CONCLUSION

The optimized nanoemulsion formulation of Azilsartan significantly enhances the pharmacokinetic properties of the drug when compared to the pure form. The improved Cmax, prolonged T1/2, higher AUC_0-T, and extended MRT suggest better drug absorption, retention, and overall bioavailability. These results highlight the potential of nanoemulsion technology as an effective strategy to improve the therapeutic efficacy of Azilsartan, offering a promising alternative to conventional drug delivery methods. Future studies should focus on further clinical evaluations to confirm these findings and explore the potential for broader applications in improving the bioavailability of other drugs.

REFERENCES

1. Garaga S, Misra NC, Reddy AVR, Prabahar KJ, Takshinamoorthy C, Sanasi PD, et al. (2015). An alternative synthesis of Azilsartan: An angiotensin II receptor blocker. Curr Issues Pharm Med Sci. 28(2):72–6.
2. Hjermitslev M, Grimm DG, Wehland M, Simonsen U, Krüger M. (2017). Azilsartan Medoxomil, an Angiotensin II Receptor Antagonist for the Treatment of Hypertension. Basic Clin Pharmacol Toxicol. 121(4):225–33.
3. Li X, Liu J, Sheng C, Chen H, Cui D, Chen G, et al. (2020). Clinical Evaluation of the Tolerability and Pharmacokinetics of Azilsartan, a Potent Angiotensin Receptor Blocker, in Healthy Chinese Subjects. Clin

Pharmacol Drug Dev. 9(4):505–13.
4. Najar AM, Eswayah A, Moftah M Ben, Omar RMK, Bobtaina E, Najwa M, et al. (2023). Rigidity and Flexibility of Pyrazole, s-Triazole, and v-Triazole Derivative of Chloroquine as Potential Therapeutic against COVID-19. J Med Chem Sci. 6(9):2056–84.
5. Ansori ANM, Ali Murtadlo AA, Kharisma VD, Muchtaromah B, Tamam MB, Rahma Turista DD, et al. (2024). Alpha-Mangostin as an Antiviral Candidate: A Mini Review. J Med Chem Sci. 7(2):275–86.
6. Kumar G, Virmani T, Pathak K, Kamaly O Al, Saleh A. (2022). Central Composite Design Implemented Azilsartan Medoxomil Loaded Nanoemulsion to Improve Its Aqueous Solubility and Intestinal Permeability: In Vitro and Ex Vivo Evaluation. Pharmaceuticals. ;15(11). 90-92
7. Bourbon AI, Gonçalves RFS, Vicente AA, Pinheiro AC. (2018). Characterization of Particle Properties in Nanoemulsions. Nanoemulsions Formul Appl Charact. 519–46.
8. Wilson RJ, Li Y, Yang G, Zhao CX. (2022). Nanoemulsions for drug delivery. Particuology. 64:85–97.
9. Khanahmadi M, Shahrezaei F, Sharifi M, Rezanejade Bardajee G. (2023). Ultrasound-Assisted Preparation and Optimization of Natural Flavoring Nanoemulsion for Dairy Products Based on Pistacia Khinjuk in Lab Scale. Chem Methodol. 7(7):524–39.
10. Varuna U, Venkata N, Vidiyala N, Sunkishala P. (2025). Bio-Inspired Green Synthesis of Nanoparticles for Psoriasis Treatment : A Review of Current Status and Future Directions. Asian J Green Chem. 9:373–403.
11. Ali HH, Hussein AA. (2017). Oral nanoemulsions of candesartan cilexetil: formulation, characterization and in vitro drug release studies. AAPS Open.3(1). 19-23
12. Sokolov Y V.(2014). Nanoemulsions as prospective drug delivery systems. Vìsnik Farm. 0(1(77)):21–5.
13. Dhahir RK, Al-Nima AM, Al-Bazzaz FY. (2021). Nanoemulsions as ophthalmic drug delivery systems. Turkish J Pharm Sci. 18(5):652–64.
14. Faheim Samar, Gardouh Ahmed, Nouh Ahmed, Ghorab Mamdouh. (2018). Review article on nanoemulsions and nanostructured lipid carriers. Rec Pharm Biomed Sci.2(2):23–31.
15. Agnihotri N, Soni GC, Kumar Chanchal D, Tiwari S. (2019). A Scientific Review On Nanoemulsion For Targeting Drug Delivery System Introduction: An ideal drug delivery system fulfills the objective of maximizing therapeutic effect while minimizing toxicity. Int J Life Sci Rev 16 IJLSR [Internet]. 5(2):16–29. Available from: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJLSR.5
16. Ulu ST, Aydoğmuş Z. (2012). An HPLC method for the determination of bisoprolol in human plasma and its application to a pharmacokinetic study. J Chromatogr Sci. 50(7):615–9.
17. Sabir AM, Moloy M, Bhasin PS. (2016). HPLC Method Development and Validation: a Review. Int Res J Pharm.;4(4):39–46.
18. Nameni A, Arghavani S, Ghamari Kargar P, Beyzaei H, Ghani M, Maleki B. (2015). HPLC Analysis of Phenolic Compounds in *Agaricus Bisporus* and *Pleurotus Ostreatus* Mushrooms: An Assessment of Their Antibacterial Capacities. Chem Methodol. 9(5):343–55.
19. Pantaleão SQ, Fernandes PO, Gonçalves JE, Maltarollo VG, Honorio KM. (2022). Recent Advances in the Prediction of Pharmacokinetics Properties in Drug Design Studies: A Review. ChemMedChem. 17(1). 89-100
20. Suryawanshi RM, Gilhotra RM, Dhakad PK, Gupta T. (2025). Formulation Design and Development of Azilsartan Nano-emulsion for the Solubility Enhancement. Int J Drug Deliv Technol.15(1):285–95.
21. Earnest Oghenesuvwe E, Daniel Lotanna A. (2014). Guidelines on dosage calculation and stock solution preparation in experimental animals' studies. J Nat Sci Res www. 4(18):2225–921.