Section: Research Paper
ISSN 2063-5346

A Study of Poorly Soluble Drug Candesartan Cilexetil Nanosuspension

Virendra Kumara[1], Dashrath Singh[1]

Department of Pharmacy, IBMER, Mangalayatan University, Aligarh

Corresponding author's email ID - virendrasul@gmail.com

ABSTRACT

Candesartan Cilexetil is refer to Biopharmaceutical classification system (BSC) class II which has low solubility as well as inadequate bioavailability. Candesartan Cilexetil nanosuspension was prepared to improve the solubility of Candesartan Cilexetil by using the antisolvent precipitation-ultrasonication method having different Solvent: Antisolvent Volume Ratio 1:15. PVP K-30 for was used as Stabilizer in candesartan cilexetil nanosuspension. Plackett-Burman design was utilized to determine which factor has the biggest impact on the quality, stability, and effectiveness of the nanosuspension. Mean particle size and saturation solubility, was assessed using 32 complete factorial design and other metrics including cumulative percentage released (CPR) at two minutes, zeta potential, polydispersity index (PDI), and percent weighted drug content was also assessed. Formulations of candesartan cilexetil nanosuspension batch CFD8 showed Mean Particle Size (nm) (Mean ± SD)(240.7 ± 8.3) and Saturation Solubility (µg/ml) (Mean ± SD)(113.03 ± 2.51). Comparison was evaluated in-vitro dissolution of both nanosuspension with marketed formulation and un-milled suspension.

Key Word: Nanosuspension, Candesartan Cilexetil, antisolvent precipitation-Ultrasonication Method

1. Introduction

Small particle sizes and large surface areas of nonosuspensions enhance the solubility and dissolution of poorly water-soluble pharmaceuticals, giving them a high commercial potential. Moreover, they can alter the pharmacokinetics of the drug, thereby enhancing its efficacy and safety. These benefits can be used to increase the bioavailability of poorly soluble medications administered orally, topically, intravenously, pulmonaryly, ocularly, or nasally for systemic or local effects. In addition to pure pharmaceuticals in aqueous media, nanosuspension's may

contain stabilizers, organic solvents, surfactants, co-surfactants, cryoprotectants, osmogents, and other substances. In nanosuspension formulations, the selection of stabilizer types, such as surfactants and/or polymers, and their ratio are the most crucial factors. nanosuspension may be administered to patients in liquid dosage forms, or post-production processes (freeze drying, spray drying, or spray freezing) may be used to transform the liquid state into the solid state for the preparation of powders, pellets, tablets, capsules, films, or gels [1].

For inadequately water-soluble drugs, solubility is a crucial factor in the development of drug delivery systems. Several conventional methods for enhancing solubility have limited applicability, particularly for drugs that are inadequately water soluble. Using nanosuspension technology, poorly water-soluble medications can have their solubility, stability, and bioavailability improved. Nanosuspensions are biphasic systems made up of purified drug particles dispersed in an aqueous medium and stabilized by surface-active agents. Nanosuspension fabrication is uncomplicated and more advantageous than other methods [2,3]. Nanosuspensions have been manufactured using methods such as high-pressure homogenization, moist milling, emulsification, solvent evaporation, bottom-up technology, and top-down technology. It is possible to administer nanosuspension via oral, pulmonary, parenteral, and ocular routes. In addition to resolving solubility and bioavailability issues, nanosuspension also improves drug safety and efficacy. In this context, we reviewed the current nanosuspension development techniques and their recent applications in drug delivery systems [2,4].

2. MATERIALS AND METHODS

Materials

Candesartan cilexetil was obtained as kind gift Sample from Alembic Research Centre, Vadodara. Poloxamer 188 and Poloxamer 407 were obtained as kind Gift Sample from Astron Research Centre, Ahmedabad. Polyvinyl alcohol was provided by Loba Chemie Pvt. Ltd., Mumbai. PVP K30 was provided by S. D. Fine Chemicals, Mumbai. Sodium Lauryl Sulphate was provided by Himedia Laboratories Pvt. Ltd., Mumbai. All the materials were used as an analytical grade.

Methods

a) Plackett-burman design (PB)

Plackett-burman design provided the interaction effects are nil or small, the Plackett-Burman design is effective for measuring main effects [3].

b) Optimization of other preliminary parameters

Candesartan Cilexetil nanosuspension

PVP K-30 was used to stabilise the candesartan cilexetil nanosuspension during preparation. We chose the three distinct PVP K-30 dosages of 30 mg, 40 mg, and 50 mg. 50 mg of PVP K-30 was chosen since it had the smallest mean particle size and highest saturation solubility. 800, 1000, and 1200 RPM were chosen to optimise the stirring speed. 1200 RPM stirring speed was selected which was showing minimum mean particle size and maximum saturation solubility. Once the precipitation of drug particle had occurred in suspension, to convert into the uniform nanosized particles probe sonicator was used. For sonication time, 10 minutes, 20 minutes, and 30 minutes of time were screened. 30 minutes of sonication duration was chosen since it produced samples with the smallest mean particle size and highest saturation solubility [4,5,6,7].

c) 3^2 factorial design

3^2 factorial design for CCNS

Using changing amounts of Candesartan Cilexetil and the solvent:antisolvent volume ratio, various formulations were created [8].

d) Evaluation of optimized batch

Evaluation parameter as well as Size of the particles and PDI, Zeta potential, Drug content, Saturation solubility and In-Vitro dissolution were performed [9].

Size of the particles and PDI

The generated nanosuspension's mean particle size and size distribution (polydispersity index) were measured using the Zetasizer (Zetatrac, Microtrac, Japan). The materials were properly diluted with water to an acceptable scattering intensity before the measurement and then re-dispersed by shaking. A nanosuspension's average particle size ranges from 200 to 1000 nm [10,11].

Zeta potential

Using the Zetasizer [Zetatrac, Microtrac, Japan] to measure the electrophoretic mobility of the particles, the Zeta Potential was evaluated in this work [12].

Drug content

The produced nanosuspension was divided into an aliquot (1 ml) and diluted with methanol before being filtered through a 0.2 m filter. A UV spectrophotometer was used to calculate the total drug content at the drug's maximum concentration [13,14].

Saturation solubility

Optioned nanosuspension's was placing in a vial and keeping it for 48 hours while stirring with a magnetic stirrer running at 100 RPM to assure saturation. An eppendorf tube containing 2 ml of nanosuspension was then filled, and it was centrifuged for 30 minutes at 10,000 RPM. After an appropriate dilution with dissolution media that served as a blank, the supernatant was filtered through a 0.2 m syringe filter and subjected to UV-visible spectrophotometer analysis at the drug's maximum concentration (UV-1800, Shimadzu, Japan) [10,15].

In-Vitro dissolution

USP 24 paddle tool was used for an in-vitro dissolving research (ELECTROLAB TDT-06P). According to Dissolution was carried out at 37 °C with the paddle speed specified. The dissolving vessels received nanosuspension that was equal to a dose of the medication. 5 ml samples were taken at intervals of 2, 4, 6, 8, 10, 15, 30, 45, and 60 minutes, and they were promptly filtered through a 0.2 m syringe filter before being spectrophotometrically examined. 5 ml of brand-new medium was then added to the dissolving vessel [11,16,17].

Table 1. Dissolution conditions for nanosuspensions

Dissolution Condition	**Candesartan Cilexetil Nanosuspension**
Dissolution media	0.05M Phosphate buffer pH 6.5, containing 0.7% v/v Polysorbate 20
Volume of Dissolution media	250
Speed in RPM	50
Sampling Intervals	2, 4, 6, 8, 10, 15, 30, 45, 60mins
Dose of drug	16mg

e) Lyophilization of optimal batch nanosuspension

Mannitol (1:1, total solid: cryoprotectant) was used as a cryoprotectant to turn the nanosuspension into a dry powder using a lyophilizer. Samples were retained in the compartment for the lyophilization process with the temperature held at -80°C for 8 hours. After being transformed into dry powder over a period of 6 to 8 hours, the nanosuspension was taken out of the chamber and put in an airtight container for further use [18].

f) Accelerated stability Study

According to ICH recommendations, accelerated stability investigations of lyophilized nanosuspension were carried out at 25+2°C and 60+5% RH for 6 months. Nanosuspension that had been lyophilized was enclosed in firm gelatin capsules. The samples were taken out and examined for particle size, saturation solubility, percent CPR at 2 minutes, and percent weight-to-weight of drug content at regular intervals (0, 1, 3, and 6 months) [19].

3. RESULT AND DISCUSION

a) Plackett-burman design (PB)

The Plackett-Burman design is useful for measuring main effects as long as the interaction effects are zero or insignificant. The fact that the chosen response parameters varied widely. suggests that the independent factors had a considerable impact on them [8].

Plackett-burman design for Candesartan Cilexetil nanosuspension

Table 2 - Layout and observed responses of Plackett-Burman design batches for CCNS (Preliminary screening formulations)

Batch Code	Amount of Candesartan Cilexetil (mg)X1	Amount of PVP K-30 (mg) X2	Solvent: antisolvent Volume Ratio X3	Stirring Speed (RPM) X4	Sonication Time (Min) X5	Saturation Solubility (µg/ml) (Mean±SD)*Y1	Mean Particle Size (nm) (Mean±SD)* Y2
CF1	20	50	1:20	800	30	95.24 ± 2.2	259.1 ± 4.7
CF2	10	50	1:20	1200	10	89.45 ± 1.9	272.2 ± 3.9
CF3	10	30	1:20	1200	30	88.25 ± 1.8	370.1 ± 6.2
CF4	20	30	1:10	1200	30	100.54 ± 2.1	257.1 ± 5.2
CF5	10	50	1:10	800	30	93.24 ± 0.9	337.1 ± 4.2
CF6	20	30	1:20	800	10	117.30 ± 0.8	218.5 ± 4.6
CF7	20	50	1:10	1200	10	75.30 ± 0.9	467.1 ± 5.9
CF8	10	30	1:10	800	10	45.54 ± 1.4	563.1 ± 6.2

*Indicates average of three determinations

Fig 1. and Fig 2. showed that the amount of Candesartan Cilexetil and the solvent to antisolvent volume ratio had the greatest impact on mean particle size and saturation solubility.

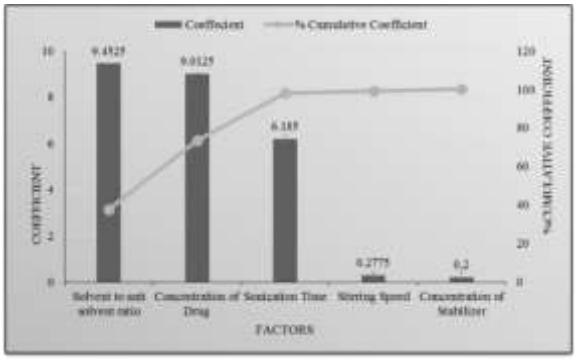

Fig 1. Pareto chart of the effect of independent variables on saturation solubility of CCNS

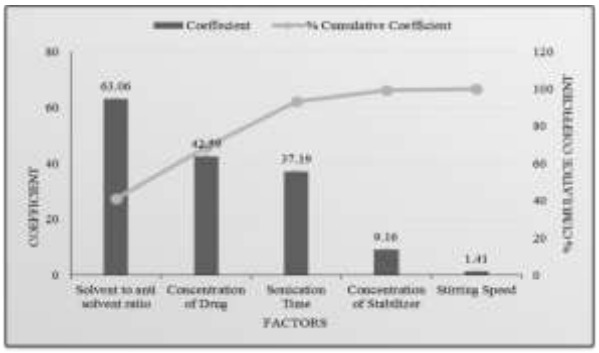

Fig 2. Pareto chart of the effect of independent variables on mean particle size of CCNS

b) Optimization of other preliminary parameters

Table 3 - Results of optimization of other preliminary parameters for CCNS

Batch Code	**Preliminary Parameters**		**Mean Particle Size (nm) (Mean ± SD)***	**Saturation Solubility (µg/ml) (Mean ± SD)***
CF9	Amount of Stabilizer (mg)	30	328.6 ± 5.2	85.18 ± 1.12
CF10		40	301.2 ± 8.3	92.26 ± 1.22
CF11		**50**	**293.6 ± 7.8**	**95.78 ± 1.15**
CF12	Stirring Speed (RPM)	800	389.9 ± 6.8	88.12 ± 0.99
CF13		1000	342.3 ± 9.9	91.43 ± 1.29
CF14		**1200**	**251.1 ± 4.7**	**102.32± 1.03**
CF15	Sonication Time (min)	10	382.9 ± 3.9	79.82 ± 1.10
CF16		20	319.1 ± 9.5	87.35 ± 1.32
CF17		**30**	**244.6 ± 6.3**	**95.4 ± 1.15**

*Indicates average of three determinations

c) 3^2 factorial design

3^2 Factorial design for CCNS

Using changing amounts of Candesartan Cilexetil and the solvent:antisolvent volume ratio, various formulations were created. The influence of both independent variables on the

predetermined dependent variables, namely mean particle size and saturation solubility, was assessed using a 3^2 complete factorial design, as shown in Table 4.

Table 4 - Layout and observed responses of 3^2 factorial design for CCNS

Batch Code	Level of Amount of Candesartan Cilexetil X1	Level of Solvent : Antisolvent Volume Ratio X2	Mean Particle Size (nm) (Mean ± SD)* Y1	Saturation Solubility (µg/ml)(Mean ± SD)* Y2
CFD1	-1	-1	435.0± 9.3	72.97±2.16
CFD2	-1	0	343.0± 6.1	95.23±2.04
CFD3	-1	1	381.0± 8.8	85.88±1.68
CFD4	0	-1	411.0± 7.1	35.99±0.62
CFD5	0	0	310.0±11.1	50.05±0.92
CFD6	0	1	354.8± 6.0	38.94±1.12
CFD7	1	-1	336.7± 7.7	94.71±1.55
CFD8	**1**	**0**	**240.8± 8.6**	**114.03±2.53**
CFD9	1	1	319.0± 9.1	101.35±1.33

Translation of Coded Levels in Actual Units			
Variables Level	**Low (-1)**	**Medium (0)**	**High (1)**
X1	10 mg	15 mg	20 mg
X2	1:10	1:15	1:20

*Indicates average of three determinations

Table 5 - Other evaluation parameters of factorial batches of CCNS

Batch Code	CPR at 2mins (% w/w) (Mean ± SD)*	PDI (Mean ± SD)*	Zeta Potential (mV) (Mean ± SD)*	Drug Content (%w/w) (Mean ± SD)*
CFD1	91.46 ± 3.85	0.540 ± 0.052	18.87 ± 1.53	93.27 ± 1.22
CFD2	99.80 ± 1.03	0.525 ± 0.050	-29.42 ± 2.13	94.83 ± 2.15
CFD3	97.22 ± 1.44	0.665 ± 0.072	16.54 ± 1.25	95.37 ± 1.36
CFD4	95.51 ± 2.02	0.745 ± 0.085	-24.37 ± 0.99	92.73 ± 0.58
CFD5	98.47 ± 3.88	0.866 ± 0.096	-10.50 ± 0.58	99.37 ± 0.87
CFD6	98.03 ± 0.84	0.877 ± 0.102	18.76 ± 1.66	99.47 ± 1.53
CFD7	99.74 ± 1.45	0.532 ± 0.086	17.19 ± 1.89	98.23 ± 1.44
CFD8	**97.24 ± 1.92**	**0.354 ± 0.043**	**25.99 ± 1.86**	**101.18 ± 1.59**
CFD9	93.01 ± 2.12	0.998 ± 0.059	22.15 ± 2.13	98.94 ± 1.28

*Indicates average of three determinations

Table 6 - Formulation and process parameters for an optimized batch of CCNS

Amount of Candesartan Cilexetil	20 mg
Amount of PVP K-30	50 mg
Solvent : Antisolvent Volume Ratio	1:15
Stirring Speed	1200 RPM
Stirring Time	4 h
Sonication Time	30 mins
Amount of lyophilizer (1:1, Total Solid: Mannitol)	70 mg

Particle size and PDI

Particle size and PDI of CCNS

Fig 3 displays the improved batch's particle size distribution. The optimized batch's mean particle size was 242.8 ± 10.20 nm, and its PDI value was 0.342

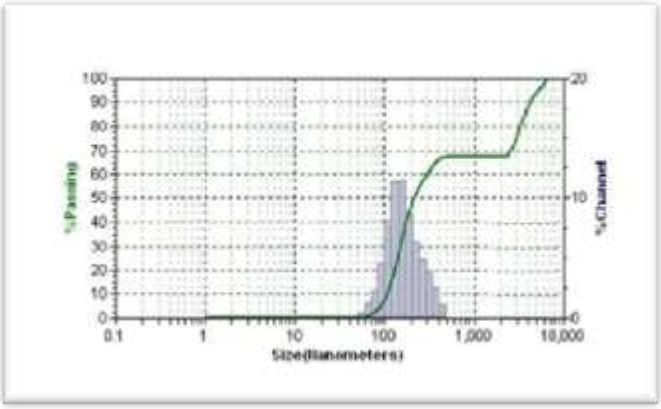

Fig 3. Particle size graph for CCNS

Zeta potential

Zeta potential of CCNS

In general, zeta potential value of ± 30mV is sufficient for the stability of nanosuspension. Zeta potential of optimized formulation was observed 25.97±1.86 mV which complies with the requirement of zeta potential.

Drug content

Drug content of CCNS

By using a UV-Visible spectrophotometer to measure the amount of drug present at 254 nm, the total amount of candesartan cilexetil was found to be 101.02% w/w.

Saturation solubility

Saturation solubility CCNS

The optimized batch of candesartan cilexetil nanosuspension's and pure drug solubility was found to be 109.7 µg/ml and 1.191 µg/ml, respectively.

In-Vitro dissolution

In-Vitro dissolution of CCNS

Fig 4 displays the dissolution profiles of the commercial formulation (ATACAND® Tablet), unmilled (pure drug) suspension, and nanosuspension. More than 97.14% of the medication was released from the nanosuspension after 2 minutes, compared to 34.64% and 75.98% at 60 minutes for the cumulative percentage release of the marketed formulation and un-milled suspension, respectively. Therefore, candesartan cilexetil's rate of dissolution was greatly accelerated by nanosuspension.

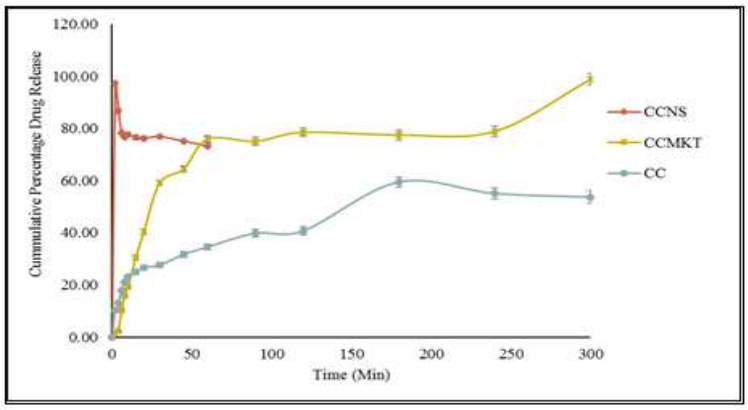

Fig 4. Comparison of in-vitro dissolution of Candesartan Cilexetil nanosuspension with Marketed formulation and Un-milled suspension

Accelerated stability study

According to the results of the accelerated stability investigation, lyophilized candesartan cilexetil nanosuspension was both physically and chemically stable when kept at 25±2°C and 60±5% RH for six months. Results from Table 7 for mean particle size, saturation solubility, cumulative percentage release at 2 minutes, and percent weight of drug content showed a little shift in all parameters with a bias of less than 5%, which was not statistically significant. The negligible difference was observed in results obtained from the optimised batch before and after the stability study according to ICH guideline.

Table 7 - Results of accelerated stability study of CCNS

Sr. No.	Storage condition for stability study	Time Period (months)	Evaluation Parameters			
			Mean Particle Size (nm) (Mean ± SD)*	Saturation Solubility (µg/ml) (Mean ± SD)*	CPR at 2mins (%w/w) (Mean ± SD)*	Drug Content (%w/w) (Mean ± SD)*
1	25°C ± 2°C and 60% ± 5% RH	0	242.6± 4.6	111.86 ±1.6	97.14±0.62	101.02±0.36
2		1	259.2± 5.2	110.51 ±0.8	96.86±2.96	100.86±0.53
3		3	265.2± 8.2	109.8 ±0.9	95.93±1.13	99.86±0.48
4		6	281.1±7.8	109.08 ±1.8	95.14±1.82	97.17±0.88

*Indicates average of three determinations

4. CONCLUSION

Candesartan Cilexetil Nanosuspension was developed by using antisolvent precipitation-ultrasonication method with help of Plackett-Burman design and 3^2 complete factorial design. Nanosuspension was evaluated by different parameters as well as Mean Particle Size, polydispersity index (PDI), Zeta Potential, Drug Content, Saturation Solubility, CPR at 2 mins. In-vitro dissolution was performed for developed Candesartan Cilexetil nanosuspension and Comparison were performed with marketed formulation and un-milled suspension.

5. ACKNOWLEDGEMENT

Author is grateful to the Dean and Guide, Department of Pharmacy, Institute of Biomedical Education And Research (IBMER), Mangalayatan University, Aligarh, for extending support to carry out the research work.

REFERENCES

1. Pınar, S. G., Oktay, A. N., Karaküçük, A. E., Çelebi, N. Formulation Strategies of Nanosuspensions for Various Administration Routes, *Pharmaceutics.* 2023;15(5):1520.
2. Azimullah, S., Vikrant., Sudhakar, C., Kumar, P., Patil, A., Usman, M. R. M., Usman, M. Z. S., Jain, B. V. Nanosuspensions as a promising approach to enhance bioavailability of poorly soluble drugs : An update, *Journal of Drug Delivery and Therapeutics.* 2019;9(2):574-582.

3. Patel, N., Patel, H. A. Application of Plackett-Burman Screening Design in Optimization of Process Parameters for Formulation of Canaglifozin Nanosuspension, *International Journal of Pharmaceutical Sciences and Nanotechnolog*y. 2020;13(6):5208-5216.
4. Dabhi, M. R., Ghodasara, U. K., Mori, D. D., Patel, K. A., Manek, R., Sheth, N. R. (2015). Formulation, optimization and characterization of candesartan cilexetil nanosuspension for in vitro dissolution enhancement, *African journal of pharmacy and pharmacology*. 2015;9(5):102-113.
5. Detroja, C., Chavhan, S., Sawant, K. K. Enhanced Antihypertensive Activity of Candesartan Cilexetil Nanosuspension: Formulation, Characterization and Pharmacodynamic Study, *Scientia Pharmaceutica*. 2011;79(3):635–651.
6. Thakkar, H. P., Patel, B. V., Thakkar, S. P. Development and characterization of nanosuspensions of olmesartan medoxomil for bioavailability enhancement, *Journal of Pharmacy and Bioallied Sciences.* 2011;3(3):426-434.
7. Liu, D., Xu, H., Tian, B., Yuan, K., Pan, H., Ma, S., Yang, X., Pan, W. Fabrication of Carvedilol Nanosuspensions through the antisolvent precipitation–ultrasonication method for the improvement of dissolution rate and oral bioavailability, *AAPS PharmSciTech.* (2012);13(1):295-304.
8. Shirsath, N., Marathe, D., Jaiswal, P., Zawar, L. A 3^2 Factorial Design Approach for Formulation and Optimization of Azilsartan Medoxomil Nanosuspension for Solubility Enhancement, *Indian Journal of Pharmaceutical Education and Research.* 2022;56(2S):S365-S373.
9. Nekkanti, V., Pillai, R., Venkateshwarlu, V., Harisudhan, T. Development and characterization of solid oral dosage form incorporating candesartan nanoparticles, *Pharmaceutical Development and Technology.* 2009;14(3):290-298.
10. Gurunath, S., Nanjwade, B. K., Patila, P.A. Enhanced solubility and intestinal absorption of candesartan cilexetil solid dispersions using everted rat intestinal sacs. *Saudi Pharmaceutical Journal.* 2014;22(3):246–257.
11. Acharya, A., B, K. K. G., Ahmed, M. G. & Paudel, S. A novel approach to increase the bioavailability of Candesartan Cilexetil by proniosomal gel formulation: in-vitro and in-vivo evaluation. *International Journal of Pharmacy and Pharmaceutical Sciences.*

2016;8(1):241-246.

12. Latha, M. M., Sundar, V. D., Kumar, S. N. & Raju, M. D. D. Design and Evaluation of Sustained Release Tablets containing Solid dispersion of Ziprasidone hydrochloride. *International Journal of PharmTech Research.* 2014;6(3):959-968.
13. Anwar, W., Dawaba, H. M., Afouna, M. I., Samy, A. M., Rashed, M. H., Abdelaziz, A. E. Enhancing the Oral Bioavailability of Candesartan Cilexetil Loaded Nanostructured Lipid Carriers: In Vitro Characterization and Absorption in Rats after Oral Administration, *Pharmaceutics.* 2020;12:1047
14. Bisht, T., Bolmal, U., Nainwal, N. Formulation of Candesartan Cilexetil Nanoparticles by Ionotropic Gelation Method Using Ultrasonication, *Ind. J. Pharm. Edu. Res.* 2023;57(3):728-735.
15. Baishya, B., rahman, S. S., Rynjah, D., Barman, K., Bordoloi, S. S., Islam, J., Hasan, N. Enhancing of oral Bioavailability of Poorly Water-Soluble Antihypertensive Drugs, *International Journal of Current Pharmaceutical Research.* 2021;13(4):42-47.
16. Xin, Z., Luk, C. L., Shirui, M. Nanosuspensions of Poorly Water Soluble Drugs Prepared by Top-down Technologies, *Bentham Science Publishers.* 2014;20(3):388-407.
17. Wang, Y., Zheng, Y., Zhang, L., Wang, Q., Zhang, D. Stability of nanosuspensions in drug delivery, *Journal of Controlled Release.* 2013;172(3):1126-1141.
18. Vijay, A., Meenakshi, B. Nanosuspension Technology: A Strategic Approach for Poorly Soluble Drugs, *Bentham Science Publishers.* 2013;3(1): 72-85.
19. Katti, V. S., Kadam, A. M., Honmane, S. M., Patil, S., Patil, S., Bhamare, K. Improvement of Solubility And Dissolution Rate of Candesartan Cilexetil By Solid Dispersion In Polyvinyl Pyrrolidone, *International Journal of Pharmaceutical science and Research.* 2014;5(4):1550-1556.