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FORMULATION AND CHARACTERIZATION OF AZILSARTAN LOADED SELF EMULSIFYING DRUG DELIVERY SYSTEM

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Abstract-

The object of the present work was the advancement and portrayal of Azilsartan stacked self-emulsifying drug conveyance framework is to increase its solubility and thereby dissolution rate and bioavailability. Self-Emulsifying Drug Delivery System was ready by the basic emulsification method. Six clumps for example F1 to F6 were ready by shifting the convergence of oils, surfactant, co-surfactant, and co-dissolvable and assessed for the different boundaries, for example, Optical microscopy, Assessment of self emulsification, Emulsification time, Droplet size investigation, Zeta Potential Measurement, Transmission Electron Microscopy, Viscosity Determination, Drug content, Percentage conveyance, in vitro disintegration study and solidness study. The SEDDS was upgraded and group F5 was additionally utilized. The medication content of chosen clump F5 was viewed as 97.65±1.37 %; it proposes that the technique for exemplification was powerful. As per in vitro study, around 55.13 % of the medication was delivered after 120 mins which showed supported

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arrival of medication and there were no critical changes seen in the actual appearance, drug content, and in vitro drug release during the stability study. The current paper presumed that the SEDDS are an expected competitor as a supported delivery drug conveyance, effectively expanding bioavailability and designated conveyance of medication.

Keywords- Self-Emulsifying Drug Delivery System, Azilsartan, Sustained release, TEM, *in vitro* release.

Introduction-

Traditional medication conveyance framework just a small part of portion ranges to fundamental blood dissemination and henceforth the majority of the portion is squandered and cause unwanted aftereffects and poisonousness and furthermore the ordinary dose structures are impacted by the gastric climate, pH conditions, and response with the stomach dividers, GI motility and presence of food in the GI parcel. Because of this the medication discharge design from measurement structure is impacted which thus influence the helpful example. This outcome in longer time of dosing, patient burden and other fundamental impacts [1]. The oral route is the most popular route among all the route of administration. Approximately 40% of new drug candidates have poor water solubility and the oral delivery of such drugs is frequently associated with low bioavailability, high intra- and inter- subject variability, and a lack of dose proportionality [2]. Recently much attention has been paid to lipid based formulations with particular emphasis on self-emulsifying drug delivery system (SEDDS), to improve the oral bioavailability of lipophillic drugs [3].

Self-emulsifying drug conveyance framework (SEDDSs) has acquired openness for their capacity to build dissolvability and bioavailability of inadequately solvent medications. SEDDSs are isotropic combinations of oils and surfactants; now and again it contains cosolvents and it tends to be utilized for the plan of details to work on the oral retention of exceptionally lipophillic compounds. SEDDS emulsify suddenly to create fine oil-in-water emulsions when brought into a fluid stage under delicate fomentation. SEDDS can be directed orally in delicate or hard gelatin cases and structure fine, moderately stable oil-in-water emulsions upon fluid weakening. [4] Expected benefits of these frameworks incorporate upgraded oral bioavailability, more steady transient profiles of medication assimilation, particular medication focusing toward a particular retention window in the GI lot, and medication assurance from the antagonistic climate in the stomach.

Azilsartan is an angiotensin II receptor blocker (ARB). It works by blocking a substance in the body that causes blood vessels to tighten. As a result, azilsartan relaxes the blood vessels. This lowers blood pressure and increases the supply of blood and oxygen to the heart. Azilsartan is a typical BCS class II medication having good permeability but poor solubility, making it an excellent option for use as a model drug in our nanostructured lipid carrier study.

In view of these contemplations, the destinations of this paper were to create Azilsartan stacked self-emulsifying drug conveyance framework for the compelling administration of hypolipidemia (RSEDDS) to upgrade the solvency and further developing bioavailability of SEDDS of Azilsartan and to manage them through oral course bringing about expanding their clinical viability.

Materials and methods-

Materials

Azilsartan was kindly provided as a gift sample by Medley Lab, Jammu, India.

Preparation of Self emulsifying drug delivery system-

This involved mixing of different oils, surfactant, co-surfactant and co-solvent shown in Table (1).

First weighed amount of Azilsartan was broken down in ethanol by constant blending in a container until it completely disintegrated. Then, at that point, a measure of oleic acid was added gradually with nonstop mixing into the drug-ethanol blend. In another beaker fitting measure of PEG-400 was added to Tween-80 and blended appropriately by ceaseless mixing with a glass pole. After nonstop blending, the combination of Tween-80 and PEG-400 was added to the medication ethanol blend by attractive mixing at 100 rpm for 30 minutes. The detailing of SEDDS was put away in a very much shut compartment for its further portrayal. [5]

Selection criteria for preparation of (F5) formulation:

The selection of formulation F5 was done on the basis of self emulsification assessment, when compared to other formulations; the F5 formulation formed a rapidly forming emulsion having a clear or bluish appearance i.e. the formulation F5 was of Grade-A preparation. (Table 2) In the above formulation design the F5 formulation is selected for the further study and characterized for various parameters.[6]

Characterization of Formulation (F5)

The opted formulation (F5) was selected and characterized for various parameters like optical microscopy, Assessment of self emulsification, Emulsification time, Droplet size analysis, Zeta Potential Measurement, Transmission Electron Microscopy, Viscosity Determination, Drug content, Percentage transmittance, *in vitro* dissolution study and stability study.

1. Optical microscopy:

The opted formulation (F5) of SEDDS observed under optical microscope (Labmed) (Fig 1)

2. Assessment of self emulsification:

The efficiency of self emulsification was assessed using standard US pharmacopoeia XXIII dissolution apparatus type II. One gm of formulation was added drop wise to 200 ml of at 37°C. Gentle agitation was provided by a standard stainless steel dissolution paddle at 60 rpm. The *in-vitro* performance of the formulation was visually assessed using the following grading system. (Table 3) [8, 9]

Grade A: Rapidly forming emulsion having a clear or bluish appearance.

Grade B: Rapidly forming, slightly less clear emulsion, having a bluish white appearance.

Grade C: Fine milky emulsion that formed within 2 minutes.

Grade D: Dull, grayish white emulsion having slightly oily appearance that is slow to emulsify longer than 2 minutes.

Grade E: Formulation, exhibiting either poor or minimal emulsification with large oil globules present on the surface.

3. Emulsification time

The emulsification time of SEDDS was determined according to USP XXIII, dissolution apparatus II. 0.5 g of the SEDDS formulation (F5) was introduced into 250 ml of 0.1N HCl in 500 ml conical flask under action of magnetic stirrer (Jaico) rotating at constant speed (50 rpm) and emulsification time was noted. (Table 4) [10]

4. Droplet size analysis

Droplet size determines the rate and extent of drug release as well as the stability of the emulsion. Formation of SEDDS, which are stable, isotropic and clear o/w dispersions, takes place on reduction of the globule size. SEDDS formulation (F5) was diluted to 100 ml with distilled water in a flask and is mixed gently by inverting the flask. The droplet size was determined by dynamic light scattering (DLS) technique using Zetasizer (Zetasizer Ver. 6.01, Malvern Instruments, (UK) (Table 5) [11]

5. Zeta Potential Measurement

The emulsion stability is directly related to the magnitude of the surface charge. The magnitude of the zeta potential gives an indication of the potential stability of the colloidal system. If all the particles have a large negative or positive zeta potential they will repel each other and there is dispersion stability. The zeta potential of the diluted SEDDS formulation was measured using a (Malvern Zetasizer 3000HS). The SEDDS were diluted with a ratio of 1:20 v/v with distilled water and mixed for 1 min using a magnetic stirrer and recorded the result. (Table 6) (Figure 2) [12, 13]

6. Transmission Electron Microscopy

SEDDS formulation F5 was diluted with distilled water 1:30 and mixed by gentle shaking. Copper grids are allowed to stand on for 60 seconds on which one drop of sample obtained after dilution was deposited. Filter paper is used to remove excess fluid and then the grid was stained in 1% phosphotungstic acid solution for 30 seconds. By following method TEM of RSEDDS was provided (Fig 3) [14, 15]

7. Viscosity Determination

Viscosity study is necessary for SEDDS to characterize the system physically and to control its stability. The viscosity of the Azilsartan SEDDS is crucial in determining its ability to be filled in hard or soft gelatin capsules. If the system has very low viscosity, it may enhance the probability of leakage from the capsule and the system with very high viscosity may create problem in pourability.

SEDDS of Azilsartan (1 ml) was diluted with the distilled water in a beaker with constant stirring on magnetic stirrer. Viscosity of the resultant emulsion and initial SEDDS was measured using Brookfield viscometer (DV-III ultra Brookfield). The data of viscosity of SEDDS formulation F5 was recorded (Table 7) [16, 17]

8. Drug content

The drug content of Azilsartan SEDDS formulation was measured using UV spectroscopic method. The drug content uniformity was determined by preparing $10 \mu g/ml$ of aliquot of SEDDS sample using methanol as solvent. The samples were suitably diluted and the absorbance of the solutions was measured at 240 nm using UV-Visible spectrophotometer (EI double beam spectrophotometer $1372 \, UV$ -Spectrophotometer) against methanol as a blank. The amount of Azilsartan was estimated by using

standard calibration curve of the drug. The data of percent drug content in SEDDS formulation (F5) was recorded in the table (Table 8) [18]

9. Percentage transmittance

Percent transmittance proved the transparency of formulation. The percent transmittance of the system is measured at particular wavelength using UV spectrophotometer (EI double beam UV-VIS spectrophotometer UV/Visible model 1372) by using distilled water as blank [19, 20]

A total of 1mL SEDDS formulation was diluted 100 times with distilled water. Percentage of transmittance was measured spectrophotometrically (EI double beam spectrophotometer 1372 UV Spectrophotometer) at 560 nm using water as a blank. (Table 9)

10. In-vitro dissolution study

The quantitative in-vitro drug discharge from detailing was contemplated to survey in the event that self-emulsifying properties stay reliable. The USP XXII, disintegration contraption (Electrolab TDT-06l) was used to concentrate on the arrival of the medication from the oil in the watery framework. Hard gelatin case containing SEDDS was attached to oar to keep the container from drifting 900 ml disintegration media were utilized standard phosphate cushion arrangement pH 7.4.

To think about various SEDDS, disintegration studies were done at 37±0.5°C, utilizing paddle pivoting at 75 rpm, 1ml example was removed at 30, 60, 90, 120, 150, 180 minutes. The example volume of new media replaces the removed example. The test was channeled through Whatman filter paper and investigated spectrophotometrically (EI twofold shaft UV-VIS spectrophotometer UV/Visible model 1372) at 240 nm. The medication discharge from the SEDDS detailing was viewed as fundamentally higher as contrasted and that of pure drug and marketed preparation. (Table 10) (Figure 4) [21]

11. Stability studies

The optimized formulations (F5), which was selected for stability testing under storage condition at 4±1°C, at 25±2°C (room temperature) and at 40±1°C (Thermostatic oven). Formulation (F5) was stored in screw capped, amber colored small glass bottles at 4±1°C, room temperature (25±2°C) and 40±1°C. Analysis of the sample was made for % drug content after a period of 15, 30, 45 and 60 days. Subsequent change in % Drug content of the formulations stored at 4±1°C, at room temperature 25±2°C and at 40±1°C (Thermostatic oven) was determined after a definite period of time of 15, 30, 45, and 60 days (Table 11, 12 & 13 and Figure 5, 6 & 7) [22]

Results-

The selection of formulation F5 was done on the basis of self emulsification assessment, when compared to other formulations; the F5 formulation formed a rapidly forming emulsion having a clear or bluish appearance that is the formulation was of Grade-A preparation. In the above formulation design the F5 formulation is finalized for the further study that is used for characterization under various parameters. (Table 2 and 3) The self emulsification assessment of SEDDS showed that the preparation was of Grade A that is a rapidly forming emulsion having a clear or bluish appearance. It was observed that an increase in the proportion of oil in the formulation resulted in decreasing self-emulsification time.

Table 2 Assessment of self emulsification for various SEDDS formulations

Formulation	Grade
F1	С
F2	В
F3	D
F4	С
F5	A
F6	В

Grade A: Rapidly forming emulsion having a clear or bluish appearance.

Grade B: Rapidly forming, slightly less clear emulsion, having a bluish white appearance.

Grade C: Fine milky emulsion that formed within 2 minutes.

Grade D: Dull, grayish white emulsion having slightly oily appearance that is slow to emulsify longer than 2 minutes.

Grade E: Formulation, exhibiting either poor or minimal emulsification with large oil globules present on the surface.

The opted formulation (F5) of SEDDS observed under optical microscope (Labmed) and it was found that the developed formulation contained the droplets in emulsion. (Figure 1)

The emulsification time of SEDDS was 19±5.51 seconds which resulted in good tendency for emulsification. (Table 4)

The droplet size of SEDDS (F5) formulation was found to be 125.89 nm which explained that the smaller droplet size presents large surface area for drug absorption. (Table 5)

The zeta potential value of SEDDS (F5) was found to be -25.7 mV negative charge indicates that the emulsion particles were stable. (Table 6) (Figure 2)

The TEM photograph shows the surface morphology of the SEDDS (F5) as seen in figure 3, the nanosized droplets as discreet particles can be seen in the TEM analysis is evidence to show that the adsorption onto solid carrier was good as no oil droplets are visible.

Viscosity of SEDDS (F5) was found to be 12.2±0.2 (cP) Thus it showed o/w emulsion where water remains as external phase and viscosity of SEDDS is near to water which indicated that Azilsartan emulsion on dilution with the fluid its viscosity getting decreased and thereby absorption will be faster. (Table 7)

The percentage drug content was found to be 97.65±1.37 which is maximum and thus resulted in maximum drug release. (Table 8)

The result of percentage transmittance was shown 97.45±1.78 (Table 9). This result indicated the high clarity of SEDDS. The greater the particle size, oil globules may reduce the transparency of micro emulsion and thereby values of percentage transmittance.

The formulation of SEDDS (F5) showed greater extent of drug release that is in 90 mins the drug released was 44.23% when compared to pure drug and marketed formulation. The results suggested the potential use of SEDDS for oral administration of Azilsartan. (Figure 4)

Accelerated stability studies only serve as tool for formulation screening and stability issues related to shipping or storage at room temperature. The results of the stability samples withdrawn at the end of 15 days, 30 days, 45 days and 60 days are shown in Table (10, 11 and 12) at various temperature ranges. All the samples withdrawn at different time intervals formed clear dispersion and none of the formulation showed any drug precipitation and thus the formulation was considered to be stable.

A progressive decrease in the emulsion % drug content has been observed in the samples withdrawn at different time intervals which may be due to aggregation of globules.

Azilsartan loaded SEDDS (F5) was subjected to stability studies. The formulation was stored at 4±1°C, at 25±2°C (Room temperature) and at 40±1°C (Thermostatic oven). From the results, it was found that % drug content had shown that formulation was more stable at 25±2°C rather than 4±1°C and 40±1°C (Thermostatic oven) storage conditions. So it can be said that the formulation is more stable at 25±2°C for further use. Table (10, 11 and 12)

Discussion- SEDDSs are isotropic combinations of oils and surfactants; now and then it contains co-solvents and it very well may be utilized for the plan of definitions to work on the oral assimilation of profoundly lipophillic compounds.

Azilsartan is a hydrophobic and exceptionally porous medication which has a place with class II of biopharmaceutical arrangement framework (BCS). Low watery dissolvability of Azilsartan prompts high changeability in ingestion after oral organization. The current review was completed for the definition improvement of Azilsartan stacked self-emulsifying drug conveyance framework (SEDDS) with the point of upgrade its dissolvability as well as oral bioavailability. The SEDDS formulation was prepared using oil components (Oleic acid), surfactants (Tween 80), Co- surfactant (PEG-400) and Solvent (Ethanol). Six formulations (F1, F2, F3, F4, F5 and F6) were developed with varying concentration of oil, surfactant and co-surfactant by simple emulsification technique and preparation (Table 1) F5 is selected for its further evaluation according to its good solubility parameters and assessment of self emulsification. (Table 2 and 3)

The self emulsifying drug delivery system of Azilsartan was characterized for its Assessment of emulsification, Emulsification time, Droplet size analysis, Zeta potential measurement, Percentage transmission, Transmission Electron Microscopy, Viscosity Determination, Drug content, In vitro dissolution study and stability study.

The opted formulation (F5) of SEDDS observed under optical microscope (Labmed) and it was found that the developed formulation contained the droplets in emulsion. (Figure 1)

The self emulsification assessment of SEDDS showed that the preparation was of Grade A that is a rapidly forming emulsion having a clear or bluish appearance (Table 2 and 3). It was observed that an increase in the proportion of oil in the formulation resulted in decreasing self-emulsification time.

The emulsification time of SEDDS was 19±5.51 seconds which resulted in good tendency for emulsification. (Table 4)

The droplet size of SEDDS (F5) formulation was found to be 125.89 nm which explained that the smaller droplet size presents large surface area for drug absorption. (Table 5)

The zeta potential value of SEDDS (F5) was -25.7 mV negative charge indicates that the emulsion particles were stable. (Table 6) (Figure 2)

The TEM photograph showed spherical surface morphology of SEDDS (F5) which resulted in higher drug loading. (Figure 3)

Viscosity of SEDDS (F5) was found to be 12.2±0.2, thus it showed o/w emulsion where water remains as external phase and viscosity of SEDDS is near to water which indicated that Azilsartan emulsion on dilution with the fluid its viscosity getting decreased and thereby absorption will be faster. (Table 7)

The percentage drug content was found to be 97.65±1.37 which is maximum and thus resulted in maximum drug release. (Table 8)

The result of percentage transmittance was shown 97.45±1.78 (Table 9). This result indicated the high clarity of SEDDS. The greater the particle size, oil globules may reduce the transparency of micro emulsion and thereby values of percentage transmittance.

The formulation of SEDDS (F5) showed greater extent of drug release that is in 90 mins the drug released was 44.23% when compared to pure drug and marketed formulation. The results suggested the potential use of SEDDS for oral administration of Azilsartan. (Figure 4) Azilsartan loaded SEDDS (F5) was subjected to stability studies. The formulation was stored at 4±1°C, at 25±2°C (Room temperature) and at 40±1°C (Thermostatic oven). From the results, it was found that % drug content had shown that formulation was more stable at 25±2°C rather than 4±1°C and 40±1°C (Thermostatic oven) storage conditions. So it can be said that the formulation is more stable at 25±2°C for further use. (Table 10, 11 and 12)

Table 1: Formulation of SEDDS

Formulations	Drug (Azilsartan) In mg	Tween- 80 in ml	PEG- 400 in ml	Ethanol in ml	Oleic acid in ml	Glycerin in ml
F1	50	3.7	-	3.6	3.7	4
F2	30	3.7	4.0	3.6	3.7	-
F3	50	3	-	4.5	3.6	4.5
F4	30	3	4.5	4.5	3.6	-
F5	50	5	2.5	2.5	5	-
F6	30	5	-	2.5	5	2.5

Table 2 Assessment of self emulsification for various SEDDS formulations

Formulation	Grade
F1	С

F2	В
F3	D
F4	С
F5	A
F6	В

Grade A: Rapidly forming emulsion having a clear or bluish appearance.

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Grade C: Fine milky emulsion that formed within 2 minutes.

Grade D: Dull, grayish white emulsion having slightly oily appearance that is slow to emulsify longer than 2 minutes.

Grade E: Formulation, exhibiting either poor or minimal emulsification with large oil globules present on the surface.[7]

Table 3 Assessment of emulsification grade (F5)

Formulation	Parameter	Observation
code		
F5	Assessment of self emulsification	Grade A

Table 4 Emulsification time of SEDDS formulation (F5)

- 1			
	Formulation code	Parameter	Result
	F5	Emulsification time	$19 \pm 5.51 \mathrm{Sec}$.

Table 5 Droplet size analysis of SEDDS formulation (F5)

Formulation code	Parameter	Result
F5	Droplet size	125.89 nm

Table 6 Zeta potential of SEDDS formulation (F5)

Formulation code	Parameter	Result
F5	Zeta potential	-25.7 mV

Table 7 Viscosity of SEDDS formulation (F5)

Formulation Code	Parameter	Result
F5	Viscosity	12.2±0.2 cP

Table 8 Drug Content of SEDDS Formulation (F5)

Formulation code	Parameter	Result
F5	% Drug Content	97.65±1.37

Table 9 Percentage transmittance of SEDDS formulation (F5)

Formulation code	Parameter	Result
F5	Percentage Transmittance	97.45±1.78

Table 10 Effect of storage on % Drug content of SEDDS at 4±1°C

Formulation	Time	% Drug Content At 4±1°C (refrigerator)
Code	(days)	
77.5	0	97.6±1
F5	15	97.6±1
	30	97.3±1
F5	45	96.9±0.2
	60	96.5±0.6

Table 11 Effect of storage on % Drug content of SEDDS at Room temperature (25±2°C)

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Formulation	Time	% Drug Content at Room temperature
Code	(days)	(25±2°C)
Code	(uays)	(23±2 C)

	0	97.6±1
F5	15	97.4±1
	30	96.8±1
	45	96.1±0.3
	60	95.7±0.7

Table 12 Effect of storage on % Drug content of SEDDS at Thermostatic temperature (40±1°C)

Formulation	Time	% Drug Content at Thermostatic temperature
Code	(days)	(40±1°C)
	0	97.6±1
F5	15	97.1±1
	13	<i>71.</i> 1±1
	30	96.5±0.5
F5	45	05.0+0.0
	45	95.8±0.9
	60	95.2±0.6

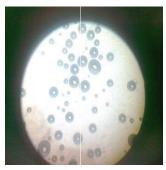


Figure 1: Photograph of formulation (F5) of SEDDS of Azilsartan under optical microscope.

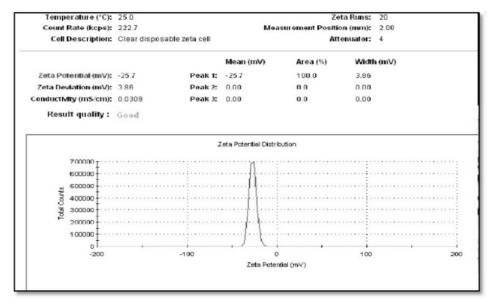


Figure 2 Zeta potential of SEDDS formulation (F5).

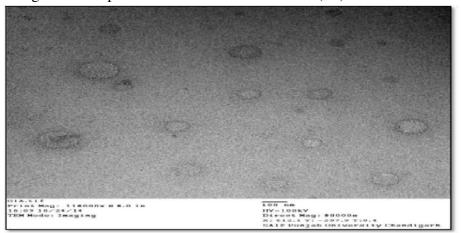


Figure 3 TEM photograph of SEDDS of Azilsartan (F5).

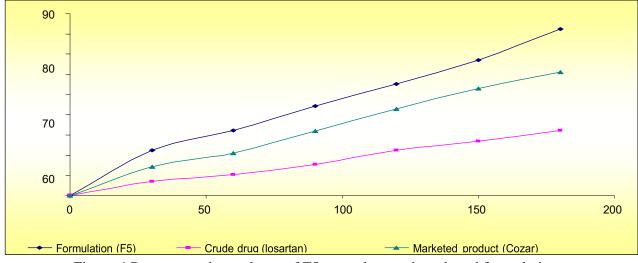


Figure 4 Percentage drug release of F5, pure drug and marketed formulation

Conclusion

All in all the current exploration was fundamentally pointed toward working on the dissolvability and bioavailability of in any case inadequately solvent BCS class II medication Azilsartan. The current review manages to detail a Azilsartan-based Self-emulsifying drug conveyance arrangement of an ineffective water solvent medication. SEDDS are the isotropic combinations of oil, surfactant, co-surfactant, and medication that structure oil in water emulsion when brought into watery stage under delicate unsettling.

The current examination work portrays a Self Emulsifying Drug Delivery System (SEDDS) of Azilsartan utilizing oil parts (Oleic corrosive), surfactants (Tween 80), Co-surfactant (PEG-400), and Solvent (Ethanol). Azilsartan is an HMG-CoA inhibitor with restricted water dissolvability, which represents a low and variable oral bioavailability (20%). Consequently, the fundamental goal of the study was to form SEDDS of Azilsartan to accomplish a superior disintegration rate which would additionally help in upgrading oral bioavailability.

From concentrate on it was inferred that, arranged fluid SEDDS was great self emulsification effectiveness and had globule size in nanometer range which might be physiologically steady.

The combinations comprising of oil (oleic acid) with surfactant (tween 80), co-surfactant (PEG 400) were viewed as ideal plans. Arranged SEDDS plans were tried for emulsifying properties and the resultant emulsion was assessed for evaluation of the productivity of self emulsification, emulsification time, thickness, zeta potential, rate conveyance, transmission electron microscopy, drug content, and in-vitro disintegration.

The plan was found to show a critical improvement as far as the medication discharge with above 83% arrival of medication inside 180 mins. Along these lines, Self emulsifying plan of Azilsartan was effectively evolved. The oral bioavailability of inadequately water-solvent mixtures is expanded by utilizing this detailing framework. So in the future, the SEDDS might be utilized as an essential instrument in lessening the portion size in the plan. The current review showed effective readiness of self-emulsifying drug conveyance frameworks of Azilsartan.

Future Prospects

An optimized Azilsartan stacked plan comprising of oleic corrosive, tween 80 and PEG 400 offers the benefit of good solubilization of Azilsartan. The significant restriction of lipophilic drugs with respect to their dissolvability in GIT could be overwhelmed by conveying these medications through self-emulsifying frameworks. Consequently, this original conveyance framework has made simple the conveyance of lipophilic tranquilizes orally which builds its bioavailability because of its little molecule drops.

Hence our investigations affirmed that SEDDS can be utilized as a potential option in contrast to the customary oral definition of BCS class II medications (ineffectively water solvent medications). Results further presume that SEDDS can be investigated as a potential medication transporter for disintegration improvement of Azilsartan and other insoluble medications.

Author's contribution

All authors have contributed in the studies performed and in the preparation of manuscripts.

Conflict of interest

The authors report no conflicts of interest.

Financial disclosure

No financial supports have been granted by any agency toconduct these studies.

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