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Lipid-Based Solid Dispersions of Azilsartan Medoxomil with Improved Oral Bioavailability: *In Vitro* and *In Vivo* Evaluation

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ABSTRACT

Introduction: Among the various techniques established, lipid-based solid dispersion approach is one by which the bioavailability of BCS class II drug shall be significantly increased without affecting the permeability of drug.

Aim & Objective: The main aim of the current research work is to evaluate the potential of lipid-based solid dispersions of Azilsartan Medoxomil to enhance oral bioavailability of Azilsartan Medoxomil (BCS Class IV molecule).

Methodology: ASD has been prepared using different hydrophilic lipid-based carriers such as gelucire 44/14 and gelucire 50/13 at the ratio of 1:1, 1:2 and 1:3. Pearlitol SD 200 was used as the hydrophilic carrier in all the formulations to convert the lipid-based solid dispersion to free-flowing powder. Solid dispersions were prepared by the solvent evaporation method. Prepared ASD's were evaluated for their micromeritics properties, saturation solubility, in vitro dissolution, and in vivo bioavailability in selected rats.

Results: Among the formulations prepared, formulation (ASD6) containing gelucire 50/13 (1:3), Pearlitol SD 200 as the carrier has shown enhanced drug release (98.9 ± 1.9 release in 30 minutes) and solubility (65.57mg/mL) compared to other formulations. Hence, this formulation is evaluated for comparative in vivo bioavailability in rats along with pure drug and marketed formulation (Zilarbi). It was found that relative bioavailability of ASD6 was increased by 1.11 times compared to pure drug and increased by 1.04 times compared to marketed formulation.

Conclusion: Hence, the study demonstrated that lipid-based solid dispersion technology can lead to improve the bioavailability of poorly soluble drugs like Azilsartan medoxomil significantly.

Key Words: Lipid-based, Liquid solid dispersions, Bioavailability, Dissolution, Solubility

INTRODUCTION

Till the date for the majority of the therapeutically active agents, the oral route is the major route of drug administration. Intrinsic solubility and rate of dissolution of the drug are very critical and important critical parameters for the drug oral absorption, especially for poorly soluble or insoluble drugs. Similarly, the permeability of the drug across the gastrointestinal tract is also crucial especially for highly hydrophilic drugs. Hence, dissolution is the rate-limiting step for highly lipophilic drugs and permeation is the rate-limiting steps for highly hydrophilic drugs in the process of their absorption from the gastrointestinal tract when they have administered through oral route. Due to poor solubility/limited dissolution rate and poor/limited permeability, class II and or class IV drugs suffer less bioavailability thereby decreased therapeutic effect. Several techniques have been reported to improve the solubility and dissolution properties of poorly soluble drugs which intern can improve absorption and bioavailability.[1,2].

Among the various techniques established, solid dispersion approach is one by which the bioavailability of BCS class II drug shall be significantly increased. This technique is the most promising approach and has been proved to improve the bioavailability of poorly soluble drugs by several research scientists[3,4,5]. It was also reported that lipid-based solid dispersion shall be prepared with using lipid-based hydrophilic carriers by which the bioavailability of class II drugs have been increased by improving the drug dissolution

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rate and bioavailability of class IV drugs have been increased by improving both the dissolution and permeability.[6-10]

Azilsartan Medoxomil, novel prodrug, is a selective angiotensin II receptor blocker that is approved in the treatment of hypertension. As it is a prodrug, it undergoes rapid de-esterification in the GIT before its absorption and produces its active form Azilsartan. Though it is administered as a prodrug, still the drug is having very less oral bioavailability in healthy humans as the drug belongs to BCS class IV and due to unfavourable breakage of the ester drug to a poorly permeable parent molecule in the gastrointestinal fluids.[11-15]

Hence, the objective of the present work was to enhance the solubility, dissolution rate and permeability and hence the oral bioavailability of poorly soluble BCS Class IV drug, Azilsartan medoxomil using lipid based solid dispersion approach.

MATERIALS AND METHODS

Materials

Azilsartan medoxomil gift sample was provided by Alembic Pharma Ltd,, Baroda, India. All the other materials were received as samples from Abitec Corp., USA.: Gelucire 44/14 and Gelucire 50/13 were received as gift samples from Gattefosse. Pearlitol SD 200, were received from Signet, India.

Saturation solubility studies

Saturation solubility studies of Azilsartan medoxomil was done in various buffers and non-volatile liquid vehicles. Excess amount of drug was added to two mL of each solvent in a screw-capped vial and was kept on the isothermal mechanical shaker at 25°C for 72 hours. After equilibrium/saturation, each solution was centrifuged at 5000 rpm for 30 minutes. The supernatant was collected and it was filtered through 0.45µ membrane filter, suitably diluted with methanol and then absorbance was measured at 249nm using UV spectrophotometer.

Phase solubility studies: Phase solubility studies were done by taking different concentrations of Gelucire 44/14 and Gelucire 50/13 (1%, 3%, 5%, and 7%) in distilled water. To each of these concentrations, the excess amount of drug was added. Then these solutions were kept for shaking on shaker for 48 hours. After 48 hours samples were filtered through the Whatman filter paper then the solution diluted and estimated for Azilsartan concentration using UV-spectroscopy. Three determinations were carried out for each sample to calculate the solubility.

Preparation of Lipid-Based Solid Dispersions

Lipid-based solid dispersions of Azilsartan were prepared by using different hydrophilic lipid-based carriers such as gelucire 44/14 and gelucire 50/13 in different ratios such as 1:1,1:2 and 1:3. These ratios were decided based on the results obtained in phase solubility studies. Pearlitol SD 200 was used as inert carrier in all the formulations. Compositions of various formulations are given in Table 1. Lipid-based solid dispersions (LBSD) of Azilsartan were prepared by the solvent evaporation method. Azilaratn 40 mg was taken and dissolved in 10 ml solvent mixture of ethanol and dichloromethane. To the drug required quantity of lipid-based carrier and the porous inert carrier was added. This solution was taken into the round bottom flask, attached to the rotary flash evaporator and evaporated at 37°C, rpm was 60 for 15 min. Solid dispersions were obtained, collected and dried in the desiccator till it was completely dried.

Evaluation of formulations

Flowability of prepared formulations was detected by Carr's index, angle of repose, and Hausner's ratio.

Angle of Repose

Angle of repose of all the prepared formulations were detected by using below provided formula:

$$\theta = \mathrm{Tan}^{-1}\,(h/r)$$

Where 'h' and 'r' are the height and radius of powder cone.

Compressibility Index

Carr's Compressibility index was calculated using below mentioned formula:

$$\text{Carr's index (\%)} = \frac{[(\text{Tapped density} - \text{Bulk density}) \times 100]}{\text{Tapped density}}.$$

Hausner's Ratio

Hausner's ratio was calculated from the equation:

Hausner's Ratio = Tapped Density/Bulk Desnity

Content Uniformity

Weight equivalent (200mg) to one unit dose of Azilsartn medoxomil (40mg) of each prepared formulation was transferred to 100mL volumetric flask, 10 mL of methanol was added and shaken for 10min and volume was made to 100mL with 6.8pH buffer. The solution was filtered, diluted suitably, and analyzed spectrophotometrically using UV-visible double-beam spectrophotometer (UV1800, Shimadzu, Japan).

Saturation Solubility Studies

Saturation solubility studies of all the prepared formulations were done in water using the same method as mentioned above

In-Vitro Drug Release Study

In vitro dissolution studies of all the prepared formulations were done using USP Type-II dissolution Paddle apparatus using 6.8pH Phosphate buffer as dissolution medium at 50rpm at 37°C ± 0.5°C. At each predetermined time intervals, 10 mL samples were withdrawn and replaced with fresh dissolution media. Upon filtration, through a 0.45 µm membrane filter and suitable dilution each sample was analysed using UV spectrophotometer.

In Vivo Pharmacokinetic Studies

The research protocol of animal experimentation was approved by the Institutional Animal Ethics Committee (IAEC Number: 516/01/A/CPCSEA). The pharmacokinetic evaluation was done in healthy Wistar rats. The animals were housed in metallic cages with free access move and provided standard laboratory diet and water in central animal house. Based on the evaluation results of in vitro dissolution studies ASD3 that showed highest drug release within 30 minutes was selected to carry out *in vivo* performance in comparison with the pure drug solution (APD) and marketed formulation Zilarbi tablets. The animal dose was calculated using the following formula.

$$Animal Equivalent Dose\ (mg/kg) = Human Dose\ (mg/kg)\ X Km value of animal\ /Km value of human$$

Km value of Rat is 7

Km value of Human is 37

As per the above formula dose of each formulation administered was 3.5mg. Each formulation was prepared by taking the weight equivalent to 3.5 mg of drug and dispersing in 1% Sodium CMC Suspension. All formulations were administered to animal through the oral cannula.

An open-label, balanced, randomized, three-period, three-treatment, three-sequence, single-dose crossover study design in which six healthy Wister rats received one treatment (product) each with a washout period of 7 days such that all products are tested in all the six healthy rats during the study. Six healthy rats with a bodyweight range of 200-250g were selected through physical examination. Animals were randomly divided into three groups consisting of two in each group. Each group on three different periods received a single dose of each of the above three treatments in random order with a washout period of one week between each treatment and the scheme of administration of the treatments is shown below table 2.

The animals were fasted overnight before administering the dose. After collecting the zero hour blood sample (blank), a standardized diet was given in the morning.

During each period, 0.5 mL venous blood samples were collected from the retro-orbital vein of each animal in Accuvet tubes (Quantum Biologicals Pvt. Ltd., Chennai, India) containing K3EDTA. Blood Samples were collected at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 18 and 24 hrs. Plasma was immediately separated by centrifugation at 5000 rpm for 30 min from the blood samples and stored in frozen conditions at -20°C with appropriate labelling of the subject code number, study date and collection time before analysis. The concentration of Azilsartan in plasma samples was measured by the HPLC method using Waters symmetry C18 (4.6 × 250 mm, 5 µm) column as column, 25 mM ammonium acetate buffer (pH 5.5): acetonitrile 55 : 45 v/v as isocratic mobile phase and UV detector at 254nm. Pharmacokinetic parameters such as peak plasma concentration (Cmax), time at which Cmax occurred (Tmax), area under the curve (AUC), elimination rate constant (Kel), and biological half-life (t½) were calculated in each case using the data by Kinetics Pro 2.0 (PK Solver 2.0) software using the non-compartmental approach. Percent relative bioavailability of the optimized formulation vs pure drug suspension and the marketed formulation was also estimated to understand the improvement of oral bioavailability with the selected method.

RESULTS AND DISCUSSION

Saturation Solubility Studies

Solubility data of drug Azilsartan medoxomil in various liquid vehicles is shown in Figure 1. Azilsartan appears to be more soluble in alkaline pH than acidic pH.

Phase Solubility Studies

Phase solubility studies were performed in different concentrations of gelucire 44/14 and 50/13 and the values obtained were represented in bar chart which is shown in Figures 2. It was observed that the phase solubility of the drug is increased with the increased concentration of the carrier.

Preparation of Lipid-Based Solid Dispersions.

Lipid-based solid dispersions of Azilsartan were prepared by using different hydrophilic lipid-based carriers such as gelucire 44/14 and gelucire 50/13 in different ratios such as 1:1,1:2 and 1:3. These ratios were decided based on the results obtained in phase solubility studies. Pearlitol SD 200 was used as inert carrier in all the formulations. Compositions of various formulations are given in Table 1

Evaluation of Formulations

Micromeritics Parameters

Good flow is required and is crucial for content uniformity. The results of various flow parameters are shown in Table 3.

All the formulations have shown good and improved flow compared to pure drug. This could be probably due to the presence of pearlitol.

Content Uniformity

All the prepared formulations were found to uniform in their drug content

Saturation Solubility studies

Results of saturation solubility studies are shown in figure 3. It was observed that all the prepared formulations have shown improved solubility in water and the formulation prepared with gelucire 50/13 carrier at 1:3 ratio (ASD6) has a comparatively higher solubility than other formulations.

In Vitro Dissolution Studies

The dissolution profiles of the pure drug, marketed formulation and lipid-based solid dispersions formulations are shown in Figures 4. It was observed that the dissolution rate has been increased significantly compared to the pure drug formulation and marketed formulation. Formulation ASD6 has shown the highest drug release in 30 minutes. Improved drug dissolution might be due to the high intrinsic solubility of the drug in the presence of gelucire 50/13 and hydrophilic coat formation of Pearlitol SD200 surrounding the drug.

In Vivo Pharmacokinetic Study

In vivo studies revealed that the bioavailability of optimized formulation is high compared to pure drug and marketed product. The plasma concentration data were given in table 4. The pharmacokinetic parameters were calculated and listed in table 5. Plasma concentration-time profile is shown in figure 5. It was observed that the oral bioavailability of optimized formulation is increased by 1.11 times and 1.04 times when compared to pure drug and marketed formulation respectively.

CONCLUSION

In the present study, the potential of lipid-based solid dispersions to improve the bioavailability of water-insoluble drug Azilsartn Medoxomil was investigated. The results showed that saturation solubility, dissolution, and in vivo bioavailability has been increased to a greater extent. Thus lipid-based solid dispersion technology shall be used to improve the bioavailability of poorly water-soluble drugs that will make the dosage form will be cost-effective.

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Table 1: Composition of Lipid Based Solid Dispersions

Name of the Ingredient	ASD1	ASD2	ASD3	ASD4	ASD5	ASD6
Azilsartan Medoxomil API	40	40	40	40	40	40
Gelucire 44/13	40	80	120	--	--	--
Gelucire 50/13	--	--	--	40	80	120
Pearlitol SD 200	120	80	40	120	80	40
Total Unit Weight (mg)	200	200	200	200	200	200

Table 2: Three way crossover treatment study for the selected formulations

	Sequence 1		Sequence 2		Sequence 3
Group 1	ASD6	WASH OUT	Zilarbi	WASH OUT	APD
Group 2	Zilarbi		APD		ASD3
Group 3	APD		ASD6		Zilarbi

Table 3: Results of Formulations

Parameters	Formulations						
	Pure Drug	ASD1	ASD2	ASD3	ASD4	ASD5	ASD6
Bulk Density	0.35	0.57	0.63	0.73	0.61	0.62	0.75
Tapped Denisty	0.66	0.62	0.74	0.77	0.66	0.68	0.84
Angle of Repose (º)	38.7	23.2	17.5	17.5	17.7	19.3	19.4
Carr's Index	47.0	8.1	14.9	5.2	7.6	8.8	10.7
Hausner's Ratio	1.9	1.1	1.2	1.1	1.1	1.1	1.1
Assay (%)	100.1	99.2	99.6	99.4	99.6	99.7	99.8
Saturation Solubility in Water (mg/mL)	0.08	15.54	32.24	45.56	22.56	48.87	65.57

Table 4: Plasma Concentration Profile of Azilsartan Formulations

Time (hrs)	Plasma Concentration (ng/mL)		
	APD	Zilarbi	ASD6
0	0.00	0.00	0.00
0.5	63.73	86.77	121.08
1	179.48	184.60	272.40
1.5	348.21	374.87	431.91
2	581.15	604.44	696.77
3	770.80	786.57	836.52
4	984.01	1010.06	1111.65
6	817.38	861.11	897.08
8	762.13	784.62	838.14
10	717.48	722.08	783.98
12	653.84	672.74	733.59
18	550.19	554.57	593.96
24	446.90	462.91	497.14

Table 5: Pharmacokinetic Parameters of Azilsartan Formulations

Parameter	Unit	APD	Zilarbi	ASD6
K	1/h	0.033	0.031	0.033
t1/2	H	20.82	22.24	20.95
Tmax	H	4	4	4
Cmax	ng/ml	984.01	1010.06	1111.64
AUC 0-t	ng/ml*h	14829.55	15220.82	16467.18
AUC 0-inf_obs	ng/ml*h	28254.77	30079.76	31493.47
AUMC 0-inf_obs	ng/ml*h^2	894295.40	1006141.38	1000534.49
MRT 0-inf_obs	H	31.65	33.44	31.76
Vz/F_obs	Ltrs	0.318	0.320	0.287
Relative BA with APD				111.46
Relative BA with Zilarbi				104.70

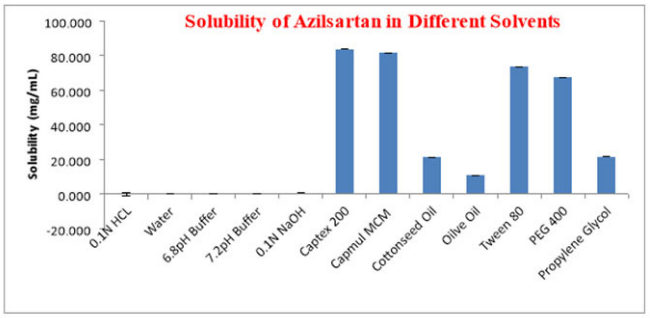

Figure 1: Solubility of Azilsartan in different solvents.

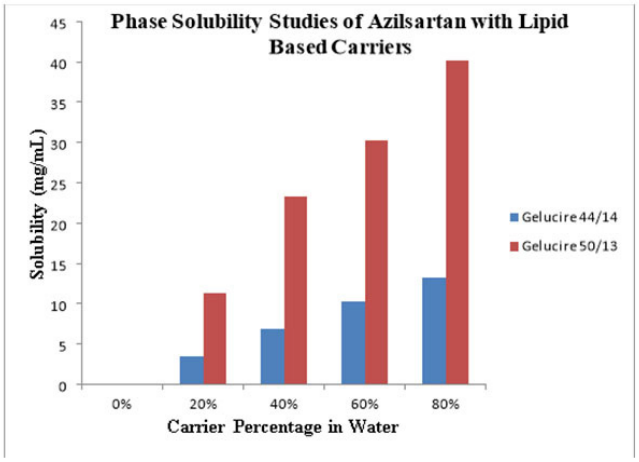

Figure 2: Phase Solubility Study Results of Azilsartan.

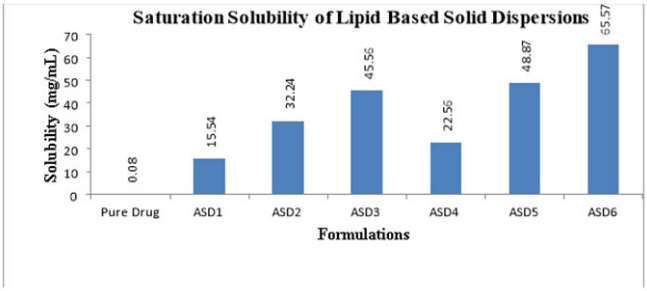

Figure 3: Saturation Solubility results of Lipid based solid dispersions in water.

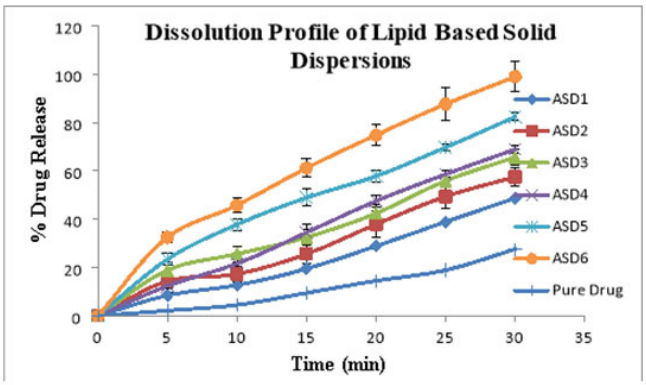

Figure 4: Dissolution Profile of Lipid Based Solid Dispersions.

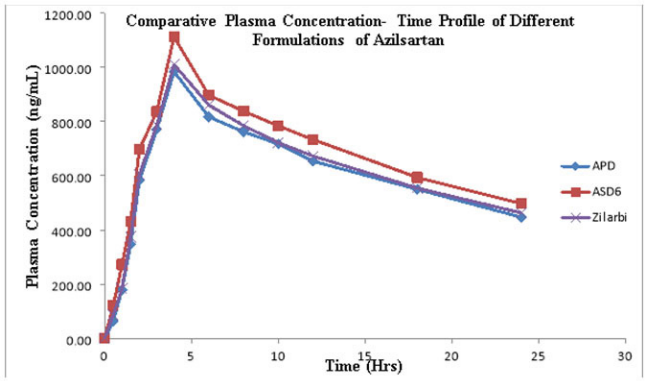

Figure 5: Comparative Plasma Concentration- Time Profile of Different Formulations of Azilsartan.