

## FORMULATION AND CHARACTERIZATION OF NOVEL SELF MICRO EMULSIFYING DRUG DELIVERY SYSTEM OF GLIMEPIRIDE

### ABSTRACT

The purpose of preparing Self microemulsifying drug delivery system in this work is to enhance the solubility and oral bioavailability of poorly water soluble drug, Glimepiride. SMEDDS are the isotropic mixture of surfactant, co-surfactant and oil incorporated with drug. In the aqueous media, gastro intestinal motility emulsification takes place. Glimepiride was undergone solubility studies in various surfactants, co-surfactants and oils. Pseudo ternary titrations were done using surfactants/co-surfactants and oil in different ratios against water. By these titrations pseudo ternary phase diagrams are constructed through which self emulsifying region are identified Tween 80 and Transcutol as surfactant and co-surfactant with sunflower oil are identified as optimized SMEDDS formulation for Glimepiride. FTIR analysis was done for investigating the drug excipient compatibility. Poorly water soluble new drug candidates are approximately 40%, whose oral delivery is frequently associated with implications of low bioavailability, high intra and inter subject variability and lack of dose proportionality. These drugs come under hydrophobic nature which is often dissolved in SMEDDS.

**Key words:** SMEDDS, Glimepiride, Tween 80, Transcutol, Phase diagrams.

### INTRODUCTION

One of the modern oral hypoglycaemic agent is Glimepiride (1- [P-2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido) ethyl] phenyl] sulfonyl]-3- (trans-4-methylcyclohexyl) urea Which belongs to the group of sulfonyl derivatives which is used for the treatment of non-insulin dependent diabetes mellitus(NIDDM) to achieve exact control of blood glucose level. Instead Glimepiride shows the principal mechanism of action with other drugs of this class like the stimulation of insulin secretion from pancreatic  $\beta$ -cell, it has multiple pharmacological benefits<sup>1,2,3</sup>. Glimepiride is the first III generation sulphonyl urea. It is a very potent sulphonyl urea with long duration of action. Chemical formula of Glimepiride drug is  $C_{24}H_{34}N_4O_5S$ .

Glimepiride is a poorly water soluble and highly permeable active pharmaceutical product belonging to BCS classII<sup>4</sup>. To improve the solubility, chemical stability and oral bioavailability of several poorly water soluble drug, self microemulsifying drug delivery systems have been successfully emerged in recent years. The SMEEDS are isotropic mixtures of oils, surfactants, co-surfactants incorporated with drug.these SMEDDS mostly forms oil-in-water microemulsions whose size would be less than 100nm(i.e. <100nm-50nm). This droplet size can be measured when this microemulsion undergoes gastrointestinal tract<sup>5,6</sup>. The large interfacial surface area is provided due to small droplet size of microemulsion which is useful for drug release and absorption<sup>7,8</sup>.

The purpose of the work done was to develop a SMEDDS formulation of Glimepiride to improve its oral bioavailability. Glimepiride was tested for its solubility behavior in various vehicles and an optimized SMEDDS containing Glimepiride was formulated. To evaluate the improved solubility and dissolution profile of Glimepiride loaded SMEDDS in comparison with 2mg glimepiride marketed tablet, dissolution was performed. The pharmacokinetics of Glimepiride loaded SMEDDS and Glimepiride suspension after oral administration in male wistar rats had been characterized and compared in order to evaluate the increased bioavailability of formulated SMEDDS. The plasma concentration of formulated SMEDDS and pure drug suspension was simultaneously determined by a modified HPLC method.

## MATERIALS AND METHODS

### Materials

Glimepiride was kindly gifted by Hetero Labs Pvt Ltd, Hyderabad, Cremophor EL was kindly donated by BASF Corp Germany, Labrasol and Transcutol was a gift sample from Gattefosse Corp France, Tween 80, Tween 20, Sunflower oil, Sesame oil, Span 80, PEG 400, Propylene glycol, Sesame oil, Brij 35 were purchased from S.D fine chemicals, Mumbai.

### Solubility studies

The solubility of Glimepiride in various oils, surfactants and co-surfactants was measured respectively. An excess amount of Glimepiride(50mg) was added into each vehicle and is stirred continuously for 48 hours at 37°C under Orbital shaking incubator(Remi electrotechnic ltd) to facilitate solubilisation. The mixtures were centrifuged at 12,000rpm for 20mins after equilibrium was achieved. The supernatant was diluted appropriately and the concentration of Glimepiride in the solution was assayed by U.V spectroscopy at 230nm.

### Construction of Pseudo ternary phase diagrams

The relation between composition of a mixture and its phase behavior can be known with construction of phase diagram<sup>9</sup>. Pseudo ternary phase diagrams were developed by water titration method against oil, surfactant and cosurfactant. The weight ratio of surfactant and co-surfactant(km) was differed as 1:1, 1:2, 2:1, 1:3, 3:1 respectively. Oil and surfactant/co-surfactant mixture mixed thoroughly in various weight ratios(i.e. 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1) respectively. Water was added slowly drop by drop using 100µl micropipette under vigorous stirring on cyclometer at 37°C until the mixture was obtained clear through which the concentrations of the components were recorded.

### Formulation optimization of Glimepiride loaded SMEDDS

Formulation of Glimepiride loaded SMEDDS was very easy by mixing the Glimepiride drug, oil, surfactant and co-surfactant by stirring under magnetic stirrer at optimum temperature. After drug dissolves the mixture appears to be in single phase which represents SMEDDS formulation loaded with Glimepiride drug. This formulation should be cooled to ambient temperature. The obtained formulation is sonicated for 48 hrs. The final formulations are equilibrated for 48hrs for the examination of turbidity or phase separation. The formulations obtained should be in single phase without any precipitation and phase separation. In all the formulations the drug amount of Glimepiride was kept constant at 2mg/ml as seen in table 1.

Formulation	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
% Tween 80	54.1	50.8	51.6	55.4	50.5	55.2	54.4	56.2	56.1	54.7
% of transcutol	27	30.4	25.2	22	23.9	17.6	17.2	32.5	12.3	11.3
% of sunflower oil	18	18.6	23	22.4	10.5	27.1	28.3	31.5	31.4	33.9

**Table 1: Formulations of Glimepiride SMEDDS.**

## **Characterization for Glimepiride loaded SMEDDS**

Droplet size analysis studies, zeta potential, TEM, SEM, self emulsification time, visual observation, cloud point measurement, FTIR, invitro drug release studies, exvivo drug release studies were carried out for Glimepiride loaded SMEDDS.

### **Determination of self emulsifying time**

The foremost means of self microemulsification assessment is through visual evaluation. 100ml water and 0.1N HCl solution is taken as medium which is stirred under magnetic stirrer with 100rpm at  $37\pm 0.5^{\circ}\text{C}$  and pour 1ml of formulated SMEDDS into the medium and the contents being mixed gently at 100rpm and determining the time required to form microemulsion upon dilution of SMEDDS with water<sup>10,11</sup>.

### **Visual observation, phase separation**

200ml of distilled water is taken in a beaker and each Glimepiride loaded SMEDDS formulation was dropped into water and this is maintained at  $37^{\circ}\text{C}$ , and the diluted preparation was vortexed for 1min. This preparation was stored for 24 hours and then phase separation and precipitation can be observed visually. Mixtures exhibiting a negligible phase separation during the 2 hr period were used for subsequent studies. It gives the information about stability and viability of the formed microemulsion<sup>12</sup>.

### **Cloud point measurement**

The formulated SMEDDS was diluted with 50ml water in a beaker which is placed on a water bath with gradually increasing temperature until the diluted formulation turned cloudy. It mainly insists about the stability of microemulsion at body temperature<sup>13</sup>.

### **Droplet size analysis (Zeta sizer)**

The Glimepiride SMEDDS was diluted with distilled water and stirred slowly under magnetic stirrer to mix thoroughly. Zeta sizer was used to determine the droplet size of resulting microemulsion. Size of droplet is measured by photon correlation using Malvern zeta sizer.

### **Transmission electron microscopy (TEM)**

SMEDDS 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 the above method which is transmission electron microscopy(TEM)<sup>14</sup> the morphology of Glimepiride loaded SMEDDS was provided by giving information on the porosity and microstructure.

### **Scanning electron microscopy(SEM)**

Optimized solid SMEDDS formulation was mounted on the stub and coated with gold particles and photomicrographs were taken at accelerating voltage of 10kV, by using LV-SEM 5800 (JOEL, Japan).

### **Fourier transform infra red spectroscopy(FTIR)**

Fourier transform infrared studies for SMEDDS can be known taking spectra of FT-IR. (Bruker-alpha T)FTIR is used to determine any type of chemical interactions between the drug and excipients<sup>15</sup>.

### **Invitro drug release studies**

Invitro drug release studies were obtained using USP dissolution apparatus typeII. 900ml of 6.8 p<sup>H</sup> phosphate buffer was placed in the dissolution vessel and the SMEDDS formulation was filled into hard gelatin capsule and placed in the dissolution medium and was stirred at 50rpm at 37°C.5ml of samples were withdrawn at pre determined time intervals of 5,10,20,30,40,50,60,70,80 and 90 minutes. And the drug concentration was determined under UV spectrophotometer at 230nm wavelength. The withdrawn volume of samples was replaced by fresh dissolution medium every time. Cumulated released amounts were plotted as a function of time<sup>16</sup>.

### **Ex vivo intestinal diffusion studies**

The institutional animal ethics committee approved protocols were followed, male wistar rats were sacrificed and intestine was collected and used for diffusion study. The intestine was suspended in phosphate buffer solution and studies were done for 8 hrs, with sampling at intervals sufficient to estimate the amount of drug diffused by using U.V. spectrophotometer.

### **In vivo studies**

Optimised glimepiride SMEDDS were tested for their bioavailability enhancement in rats, healthy male wistar rats were taken and divided into two groups, one group was treated with plain drug suspension, other was administered SMEDDS formulation of glimepiride at a dose of 1mg/kg, after successful plasma spiking of the drug, plasma samples were withdrawn by retro orbital puncture at intervals of 0.5, 1, 2, 4, 6, 8, 12, 24hrs. The plasma was treated with acetonitrile and centrifuged at 25000rpm and supernatant injected into HPLC using C 18 column, 80:20 ratio of methanol and 10mM phosphate buffer adjusted to pH 3, at 220nm of wavelength.<sup>17,18</sup>

### **Stability studies**

Stability studies for the SMEDDS formulation was studied for its physical and chemical aspects, formulations were stored for a period of 3 months at 40±2°C/75%RH. Sampling was done at a frequency of monthly once and evaluated for drug release and particle size.<sup>19,20</sup>

### **Results**

Solubility of the drug plays a vital role in the formulation of SMEDDS. Glimepiride was solubilised in various oils, surfactants and co-surfactants whose results were given below in the graph.

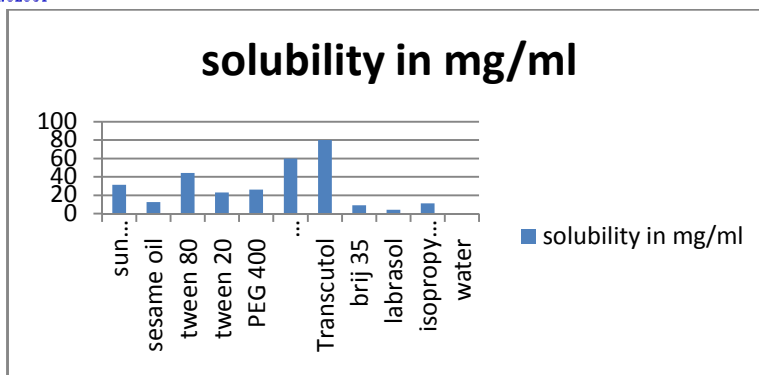


Fig1:Solubility studies of glimepiride in various vehicles.

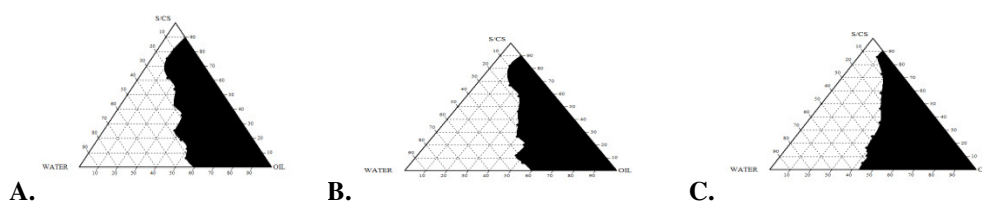


Fig 2: Pseudo ternary phase diagram of sunflower oil with tween 80: transcutool A(1:1), (1:2),(2:1)

Formulation code		F5	F6	F7	F8	F9	F10
0.1N HCl	Emulsification time(secs)	23	28	29	45	67	26
	Tendency for emulsification	Good	Good	Good	Good	Good	Good
Phosphate buffer(6.8)	Emulsification time(secs)	20	31	34	50	61	22
	Tendency for emulsification	Good	Good	Good	Good	Good	Good

Table 2: Self emulsification times of SMEDDS formulation.

Formulations	F1	F4	F7	F8	F9	F10
0Hours (absorbance)	0.756	0.234	0.456	0.467	0.045	0.225
24Hours (absorbance)	0.786	0.245	0.467	0.471	0.047	0.220

Table 3: Optical clarity values of SMEDDS formulation.

Four formulated SMEDDS were subjected to droplet analysis, zeta potential and poly dispersity index. Particle size of the SMEDDS should be <100nm which was obtained for four formulations. Zeta potential values obtained are negative due to the presence of oil droplets which seems to be good as observed in values depicted in table 4 and fig 3.

Formulations	Particle Size(nm)	Zeta Potential(V)	PDI
FG7	55	-15.2	0.240
FG8	19.43	-19.8	0.332
FG9	176.1	-22.3	0.222
F10	62.32	-19.4	0.176

Table 4: Droplet size, zeta potential and zeta sizer values of SMEDDS.

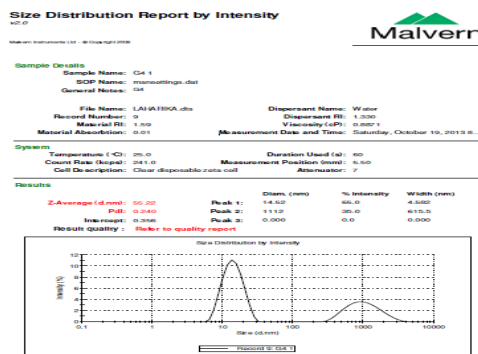


Fig 3: Droplet size analysis of SMEDDS.

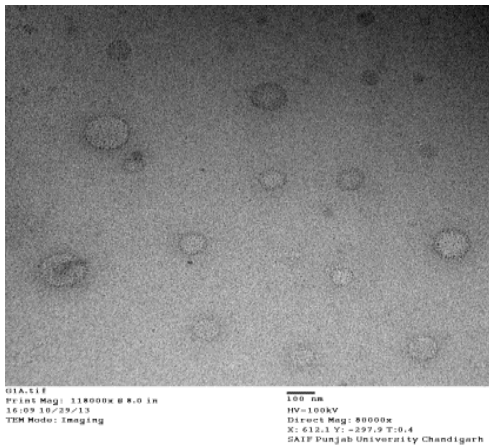


Fig 4: TEM photograph of SMEDDS

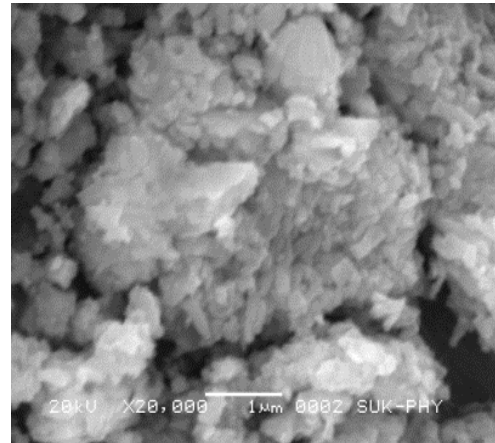


Fig 5: SEM photograph of SMEDDS

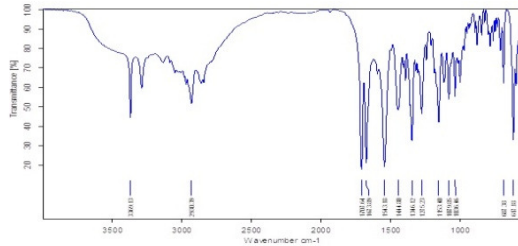


Fig 6: FTIR spectra of Glimepiride pure drug

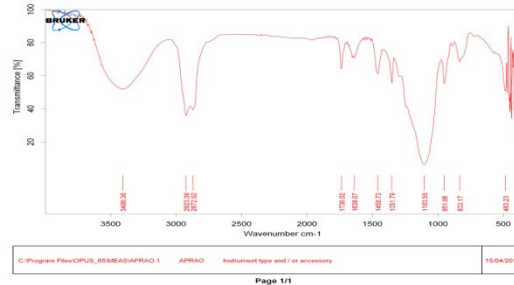


Fig7: FTIR spectra of Glimepiride SMEDDS

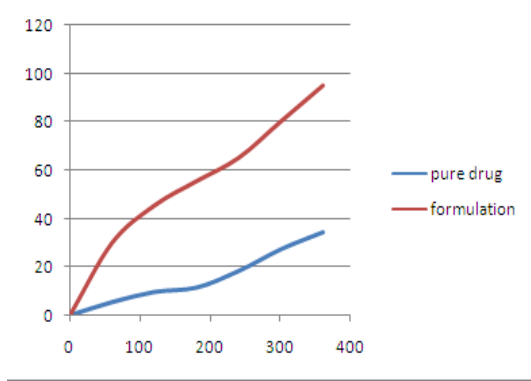


Fig 9: Ex-vivo intestinal diffusion study.

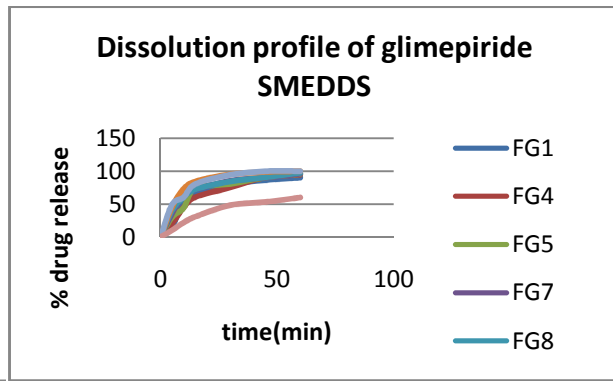


Fig 8: In vitro dissolution study of SMEDDS

The particle size analysis on SMEDDS formulations as seen in table 4, clearly demonstrate the reduction of particle size to nanometer range and efficient formation of SMEDDS, however the in vitro and ex-vivo studies also confirm the increase in dissolution due to this reason, which could be further proved by in- vivo studies.

Formulation	Cmax(µg/ml)	Tmax(hrs)	AUC tot(µg/ml-hr)
Plain drug	6.4±1.6	4.3±0.26	112.2±2.14
SMEDDS formulation	50.34±4.43	1.78±0.14	411.5±60.2

Table 5: In-Vivo studies of SMEDDS

Time (months)	Physical appearance	Drug content (%)	Particle size (nm)	PDI	Zeta potential(mV)
0	Clear liquid	100.03±0.25	62.32	0.176	-19.4
1	Clear liquid	99.94±0.32	-	-	-
2	Clear liquid	99.91±0.38	-	-	-
3	Clear liquid	99.92±0.36	60.31	0.172	-18.3

Table 6: Stability studies

## DISCUSSION

Glimepiride drug was highly solubilised in sunflower oil, Cremophore EL and Transcutol as shown in figure 1. Phase diagrams were constructed to obtain optimized concentrations of oil, surfactant and co-surfactant in the presence of Glimepiride and microemulsifying region is identified. Water/Sunflower oil/tween 80/Transcutol was shown as investigated as a quaternary system of pseudo ternary diagram which is shown in Fig 2. Formation of the emulsion was done under normal room temperature. As seen in the diagrams maximum microemulsion area was observed for phase diagrams involving sunflower oil, tween 80 and transcutol in the ratio 2:1, hence the formulations were planned using different points in this area. In the aqueous media, due to gastro intestinal motility self emulsification takes place. Six formulations are subjected to self emulsifying time test in which all the six has showed their tendency for emulsification in 0.1N HCL and phosphate buffer (6.8) in seconds who were found to be optimized. The time taken for self emulsification is shown in Table 2. Optical clarity of the selected six formulated SMEDDS was seen in 0 hours and after 24 hours. The values obtained both times were seen to be very near. The values obtained for optical clarity were shown in Table 3. No phase separation, no precipitation and clear solution of Glimepiride SMEDDS were obtained. The cloud point for the formulations was observed to be 75-80°C confirming the stability of formulation at body temperature.

The TEM and SEM photographs show the surface morphology of the optimized SMEDDS as seen in fig 4 & 5, the nanosized droplets as discreet particles can be seen in the TEM analysis and SEM photograph is evidence to show that the adsorption onto solid carrier was good as no oil droplets are visible. The FTIR spectra of the pure drug and solid SMEDDS formulation show almost similar peaks in some areas to prove absence or almost no interaction between the drug and formulation as seen in fig 6 & 7. The invitro drug release studies is observed in fig 8, the optimized formulation is seen to release 99.9% by 45 min as compared to marketed formulation which released only 60% at the end of one hour, this can be due to the high concentration of surfactants used in the formulation which has reduced the particle size of the drug to nano range hence improving the dissolution. The intestinal diffusion results can be seen in fig 9 and as seen in the drug diffusion profiles the pure drug has diffused to a very small extent of 33.6 at the end of 6 hrs whereas the SMEDDS formulation has released 80% at the end of study period. The in vivo studies in animals have demonstrated the increase in bioavailability of the formulation which can be attributed to the efficient emulsification of the microemulsion due to the surfactants used which have high HLB value, favoring the increase in solubility and dissolution. The stability studies have shown that the SMEDDS formulation was stable throughout the test period.

In conclusion the present research was mainly aimed at improving the solubility and bioavailability of otherwise poorly soluble BCS class II drug glimepiride. The phase solubility studies and the phase diagrams were helping tools in designing the optimized formulation, which was evaluated for various tests and proved to be satisfying the required parameters for effective drug dissolution and diffusion. Thus this formulation can be further tested in-vivo in animals to prove the improvement in bioavailability of the anti diabetic drug.

## REFERENCES

1. E.Drager, Diab.Res.Clin.Pract. 1995,28;139-146.
2. R.Robkamp, K.Wernicke-Panten, E.Dragaer, Diab.Res.Clin.Pract. 1996,31;33-42.
3. Petra kovarikova, Jiri klimes, Jiri dohnal, Lucie tISOVSKA. HPLCstudy of glimepiride under hydrolytic stress conditions;Journal of pharmaceutical and biomedical analysis,36(2004)205-209.
4. Amidon GL,Lennernas H,shah VP,Crison JR. A theoretical basis for a biopharmaceutical drug classification: The correlation of invitro drug product dissolution and invivo bioavailability. Pharm Res,12(1995)413-20.
5. Kang.BK, Lee Js, Chon SK, Jeong SY, YUKSH, Khang G et al. development of self microemulsifying drug delivery systems(SMEDDS) for oral bioavailability enhancement of simvastatin in beagle dogs. Int J Pharm, 274(2004)65-73.



6. Venkatesh G, Majid MI, Mansor SM, Nair NK, Croft SL, Navaratnam V. In vitro and in vivo evaluation of self microemulsifying drug delivery system of buparvaquone. *Drug Dev Ind Pharm*, 36(2010)735-45.
7. Patel D, Sawant KK. Self microemulsifying drug delivery system: formulation development and biopharmaceutical evaluation of lipophilic drugs. *Curr Drug Deliv*, 6(2009)419-24.
8. Patel D, Sawant KK. Oral bioavailability enhancement of acyclovir by Self microemulsifying drug delivery system (SMEDDS). *Drug Dev Ind Pharm*, 33(2007)1318-26.
9. Maulik J, Patel, Sanjay S, Patel, Natvarlal M, Patel, Madhabhai M, Patel. A self microemulsifying drug delivery system (SMEDDS). *International journal of pharmaceutical sciences Review and Research*, 5(2010)29-35.
10. Date AA, Nagarsenkar MS. "Novel Delivery systems of atorvastatin should be evaluated for pharmacodynamics instead of pharmacokinetics". *J.Pharm.Pharmacol*, 59(2007)1583-1584.
11. Ashok R, Patel and Pradeep R. "Preparation, in vivo evaluation of SMEDDS (Self microemulsifying drug delivery system) containing Fenofibrate". *The AAPS Journal*, 9(3) (2007)96-102.
12. Date AA, Nagarsenkar MS. "Design and evaluation of self nanoemulsifying drug delivery system (SNEDDS) for cefpodoxime proxetil". *Int.J.Pharm*, 329(2007)166-172.
13. Zhao Y, Wang C, Chow AH. "Self nanoemulsifying drug delivery system (SNEDDS) for oral delivery of Zedory Essential oil: Formulation and bio availability studies". *Int.J.Pharm*, 383(2010)170-177.
14. Mingtao Liang A, Nigel M, Davies, Istvan Totha B. "Increasing entrapment of peptides from water in oil microemulsions by copolymerization". *Int J Pharm*, 362(2008)141-146.
15. Nazzal, S, Smalyukh, I.I, Lavrentovich O.D, Khan M.A. Preparation and in vitro characterization of a eutectic based semisolid self nanoemulsified drug delivery system (SNEDDS) of ubiquinone, mechanism and progress of emulsion formation. *Int J Pharm*, 235(2002)247-265.
16. Zvonar A, Berginc K, Kristl A, Gasperlin M. "Microencapsulation of self microemulsifying systems: Improving solubility and permeability of furosemide". *International Journal of Pharm*, 388(2010)151-158.
17. Sujatha samala, sandhya rani tatipamula, ciddi veeresham. Determination of glimepiride in rat serum by RP-HPLC method. *American journal of analytical chemistry*, 2(2011)152-157.
18. Yogeshwar G, Bachhav, Vandana B, Patravale, SMEDDS of glyburide: formulation, in vitro evaluation, and stability studies. *AAPS phamScitech*. 10(2009)482-486.
19. Sanjog Jain, Amit K Jain, Milind Pohekar, Kaushik Thanki. Novel self-emulsifying formulation of quercetin for improved in vivo antioxidant potential: implications for drug induced cardiotoxicity and nephrotoxicity. *Free radical biology & medicine*. 65(2013), 117-130.
20. International conference on harmonization guidance for industry Q1A(R2): stability testing on new drug substances and products Geneva international federation of pharmaceutical manufacturers & associations: 2000.

**Hyma.P<sup>\*1</sup>, D.Laharika Reddy<sup>1</sup>, Abbulu.K<sup>2</sup>**

<sup>1</sup>Varun Herbals (DSIR-SIRO) R&D labs,  
Hyderabad, India.

<sup>2</sup>Professor & Principal, Nova college of pharmaceutical education and research,  
Hyderabad.