4. DISCUSSION

Preformulation study

The preformulation study was performed to characterize and identify the drug sample, and its possible interaction with the excipients to be used in the formulation. The identification and characterization of drug was carried out by using various analytical techniques like UV spectroscopy, FTIR and Differential Scanning Colorimetry (DSC). The wavelength of maximum absorption ($\lambda_{\text{max}}$) of the Orlistat was determined by using UV spectroscopy, which was observed to be 205 nm. FTIR analysis revealed, the presence of characteristics peak at particular wave number which confirmed existence of different functional groups in the structure of Orlistat. The Differential Scanning Colorimetry (DSC) thermo gram showed characteristic sharp melting endotherm at 42.77°C, which indicated the melting of Orlistat at particular temperature. The drug sample was identified and characterized for its different properties.

Drug-excipient compatibility study

Before formulation, it is necessary task of formulation scientist to study drug-excipient interactions to avoid possible physical and chemical incompatibilities after formulation between drug and excipients. The drug and excipients compatibility was verified by DSC and FTIR studies, which was found to be compatible. Hence, the excipients selected can be used for the further development of formulation.
Development and validation of UV spectroscopic method

The UV spectrophotometric method for Orlistat was developed and validated for different validation parameters like linearity, accuracy and precision. Developed UV spectrophotometric method was found to be simple and economic with correlation coefficient \( r^2 = 0.999 \).

Preliminary screening

Formulation of Liquisolid compact

Based on saturation solubility data non-volatile liquid vehicle with the maximum solubility was selected. Capryol PGMC was used as a non-volatile liquid vehicle for further study. Avicel PH 102 and Aerosil 200 were used as carrier and coating material.

The liquid load factor at three different level 0.1406, 0.1800 and 0.2250 was considered whereas excipients ratio at 10, 15 and 20 was considered in accordance with preliminary trial data.

Formulation of Self-emulsifying drug delivery system (SEDDS)

Ternary phase diagram was plotted to determine self-emulsifying region by conducting trials by varying concentrations of formulation components like Castor oil, Tween 80 and Capryol PGMC. The amount of oil (X1) and ratio of surfactant to co-surfactant (X2) were selected at three levels for their effect on dependent variables like globule size and emulsification time.
Formulation and Optimization LSDDS and SEDDS

Formulation of Liquisolid drug delivery system

The $3^2$ full factorial design was used successfully implemented in the present study. The effect of the independent variables were investigated at three different levels (low, medium and high) on dependent variables like angle of repose and weight of tablet. Two factors at three levels total 9 runs were generated with the help of STATGRAPHICS software. In all batches, 4% and 1% of the Sodium starch glycolate and Plasdone F-29 were added. Finally, tablets were compressed by using 10 station tablet compression machine (Rimek Mini Press-I, Karnavati) flat-faced punch and die size of 11 mm and 13 mm. From the multiple regression analysis data, it is observed that there was positive effect of the excipients ratio on angle of repose ($\beta_1 = 0.91$), % cumulative drug released at 30min ($\beta_1 = 2.08$) and Carr’s index ($\beta_1 = 0.71$). To study the effect of liquid load factor ($X_2$) on selected dependent variable regression analysis was performed. It was observed that, there was predominant positive effect of liquid load factor on angle of repose ($\beta_2 = 6.47$) which was found to be significant ($P< 0.05$) whereas no significant effect was observed on % cumulative drug released at 30min ($P>0.05$).

Formulation of Self-emulsifying drug delivery system (SEDDS)

The $3^2$ full factorial design was used successfully in the present study. Two independent variables, amount of oil ($X_1$), and ratio of surfactant to co-surfactant ($X_2$) were investigated at three levels for their effect on dependent variables like globule size and emulsification time. From the regression analysis, it was observed that there was a significant positive effect ($\beta_1 = 8.63$) of the quantity of oil on the globule size was observed, whereas predominant negative effect ($\beta_2 = -1.75$) of the
ratio of S/Co-S was observed on the globule size. It was revealed that, there was predominant positive effect of quantity of oil on the globule size ($\beta_1 = 1.16$) whereas, predominant negative effect of the ratio of S/Co-S was observed on the globule size ($\beta_2 = -1.00$).

**Evaluation of LSDDS and SEDDS**

FTIR analysis confirmed that there was no any chemical interaction has taken between drug and excipients. On XRD study, it was noticed that there was reduction in the crystallinity after formulation this might be due to solubilization effect of the drug into the solvent. The DSC analysis of liquisolid system and liquisolid system prepared with Neusilin US 2(LSN) there was disappearance of the sharp endothermic peak. It indicated that the drug was molecularly dissolved into the vehicle and it was in the form of amorphous solid solution. The % cumulative drug released at time point (30 min) was found to be higher in LS-1 to LS-3 than other batches. From all above study, LS-3 showed maximum drug release at 30min i.e. $97.3 \pm 1.92$ % than other batches. Increased lipase inhibition in developed liquisolid system LS-3 than marketed Orlistat formulation indicated that there was increased dissolution property, thereby increased amount of Orlistat availed at site of inhibition.

**SEDDS**

The globule size in all the batches (SO-1 to SO-9) of the liquid-SEDDS observed to be below 300 nm. The polydispersity index was observed in range of 0.4 to 0.6 indicated narrow size distribution of globules in the system. The minimum globule size was observed in SO-5 batch, no phase separation was observed in all formulations (SO1 to SO-9) after freeze thawing. Overall, stability of the formulation was found to be acceptable. The optimized SO-5 formulation showed spherical shape
with size range in 50–100 nm. The emulsification time was observed to be in the range of 26 ± 4 to 58 ± 4 s for all batches. It was indicated that the prepared emulsions showed rapid emulsification ability. After *in vitro* drug release study, the SO-5 formulation showed 87.37 ± 5.92% and 99.38 ±0.42 % drug release after 10min and 60min time point respectively. The optimized batch SO-5 was subjected for the freeze-drying study. The globule size of freeze dried SEDDS was found to be 110 nm ± 12 nm, which was nearly same on reconstitution. The P-XRD study showed crystalline to amorphous conversion of Orlistat in freeze-dried state. This confirmed that there was no chemical interaction occurred between drug and components used in emulsion. In case of freeze-dried SEDDS, there was no sharp endothermic peak was observed. Hence, it can be concluded that the Orlistat might be converted from crystalline to amorphous state when formulated into freeze dried SEDDS, also Orlistat might be dissolved into the excipients matrix. The porous surface of freeze dried SEDDS help to form uniform system upon reconstitution or dilution, which is due to easy penetration of wetting liquid through porous surface. Self-emulsifying Orlistat tablet showed 90 ± 2.76 % drug release after 10 min time point whereas marketed formulation showed 56 ± 2.28 %.

**In vitro lipase inhibition study**

In this study, a simple, accurate and cost effective colorimetric method with modification was developed to study enzyme inhibition, which was found to be accurate. From the determination of the linearity and regression analysis, it was found that the method was accurate and precise over the selected range. Increased lipase inhibition in developed liquisolid tablet and self-emulsifying tablet than marketed formulation indicated increased dissolution property, thereby increased amount of
Orlistat availed at site of inhibition.

**High fat diet induced antiobesity activity in Wistar rats**

From the measurement of the body weight and abdominal circumference, it was found that group treated with only high fat diet without treatment showed significant (P < 0.05) increase in body weight (326.7±7.4g) as well as abdominal circumference when compared with normal control group. The group treated with the marketed formulation (277±7.2g) showed significant increase in body weight when compared with the normal group. Lipase activity was found to be minimal in the group, which was treated with the developed formulation when compared with disease group.

**Stability studies**

Developed liquisolid tablet and SEDDS tablet passed the content uniformity test as per the USP. No significant change in drug content was observed on storage in developed liquisolid tablet and SEDDS tablet. Developed formulations showed more than 95 % of drug release after on stability study for three months.