2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Drug Profile\textsuperscript{51,52}

Drug selected for the study: Orlistat

Structural formula:

![Chemical structure of Orlistat](image)

**Fig 3: Chemical structure of Orlistat**

Chemical name: [(1S)-1-[(2S, 3S)-3-hexyl-4-oxo-oxetan-2-y1] methyl] dodecyl] (2S) - 2-formamido-4-methyl-9pentonate

Physical properties:

- **Appearance**: Crystalline solid
- **Molecular Formula**: C\textsubscript{29}H\textsubscript{53}NO\textsubscript{5}
- **Molecular Weight**: 495.5
- **Melting point**: 44 ºC
- **Solubility**: Practically insoluble in water, Approx. 20 mg/mL in ethanol.
- **Category**: Anti obesity
**Mechanism of action**

Orlistat is a reversible inhibitor of pancreatic and gastric lipases, which elicits its response in the stomach and small intestine. It forms a covalent bond with an active serine residue site of gastric and pancreatic lipases therefore the dietary fat is not hydrolysed in the form of triglycerides into absorbable free fatty acid and monoglycerides by inactive enzyme. As undigested triglycerides are not absorbed, the resulting caloric deficit may have a positive effect on weight control.

**Pharmacokinetic parameters**

The systemic absorption of orlistat is minimal, when given orally. The plasma half-life is 1 to 2 hours while > 99 % of the drug is protein bound.

**Metabolism**

Orlistat is primarily metabolized within the gastrointestinal wall forming relatively inactive metabolites. Metabolites M1 (4-member lactone) and M3 (M1 with N-formylleucine moiety) subjected for around 42% of total radioactivity in plasma. M1 and M3 have an open beta-lactone ring and extremely poor lipase inhibition activity (1000- and 2500-fold less than orlistat, respectively).

**Toxicity**

The results of a massive overdose of orlistat are unknown, although the drug seems relatively harmless.
## Marketed Preparations

**Table 3: Marketed preparations of orlistat**

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Manufacturer</th>
<th>Dosage form</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM-Slim</td>
<td>Intra Labs</td>
<td>Capsule</td>
<td>60mg, 120mg</td>
</tr>
<tr>
<td>Cobese</td>
<td>Cardiovascular (Ranbaxy Laboratories Ltd)</td>
<td>Capsule</td>
<td>60mg, 120mg</td>
</tr>
<tr>
<td>Lipophage</td>
<td>Franco-Indian</td>
<td>Capsules</td>
<td>60mg, 120mg</td>
</tr>
<tr>
<td>Nipocut</td>
<td>Neelkanth Healthcare (P) Ltd.</td>
<td>Capsule</td>
<td>60mg, 120mg</td>
</tr>
<tr>
<td>O Trim</td>
<td>Grace Drugs Pharmaceuticals</td>
<td>Capsule</td>
<td>120mg</td>
</tr>
<tr>
<td>Obelit</td>
<td>Intas Pharmaceuticals Ltd.</td>
<td>Tablet</td>
<td>120mg</td>
</tr>
<tr>
<td>Obfree</td>
<td>Bioplasma Immunological Research Pvt. Ltd.</td>
<td>Capsule</td>
<td>60mg, 120mg</td>
</tr>
<tr>
<td>Obilax</td>
<td>Strides</td>
<td>Capsules</td>
<td>60mg, 120mg</td>
</tr>
<tr>
<td>Obitrol</td>
<td>Carsyon (Micro Labs Ltd)</td>
<td>Capsule</td>
<td>120mg</td>
</tr>
<tr>
<td>Olisat</td>
<td>Biocon Limited</td>
<td>Capsule</td>
<td>60mg, 120mg</td>
</tr>
<tr>
<td>Orlica</td>
<td>Azuca (Torrent Pharmaceuticals Ltd.)</td>
<td>Capsule</td>
<td>120mg</td>
</tr>
<tr>
<td>Orlimac</td>
<td>Macleods Pharmaceuticals Pvt Ltd.</td>
<td>Capsule</td>
<td>120mg</td>
</tr>
<tr>
<td>Orlitroy</td>
<td>Troikaa Pharmaceuticals Ltd.</td>
<td>Capsule</td>
<td>120mg</td>
</tr>
<tr>
<td>Reeshape</td>
<td>Meyer Organics Pvt Ltd.</td>
<td>Capsule</td>
<td>60mg, 120mg</td>
</tr>
<tr>
<td>Slimfast</td>
<td>Intra (Intra Life)</td>
<td>Capsules</td>
<td>60mg, 120mg</td>
</tr>
<tr>
<td>Troy slim</td>
<td>Troikaa Pharmaceuticals Ltd.</td>
<td>Capsule</td>
<td>120mg</td>
</tr>
<tr>
<td>Vyfat</td>
<td>Intas Pharmaceuticals Ltd.</td>
<td>Capsule</td>
<td>120mg</td>
</tr>
<tr>
<td>Zerofat</td>
<td>Discovery (Mankind Pharmaceuticals Pvt. Ltd.)</td>
<td>Capsule</td>
<td>120mg</td>
</tr>
</tbody>
</table>

### 2.1.2 Microcrystalline cellulose

**Nonproprietary Names**

- **BP**: Microcrystalline cellulose
- **JP**: Microcrystalline cellulose
- **PhEur**: Cellulosum microcristallinum
- **USPNF**: Microcrystalline cellulose
Synonyms

Avicel PH; celex, cellulose gel; Celphere; Ceolus KG ; crystalline cellulose; E460Emcocel;Ethispheres;Fibrocel;Pharmacel;Tabulose;Vivapur.

Chemical Name and CAS Registry Number  Cellulose [9004-34-6]

Empirical Formula  C_{6}H_{10}O_{5}

Structural Formula

![Structural Formula of Cellulose]

Functional Category

Adsorbent; suspending agent; tablet and capsule diluent; tablet disintegrant.

Applications in Pharmaceutical Formulation

Microcrystalline cellulose is widely used in pharmaceuticals, primarily as a binder/diluents in oral tablet and capsule formulations where it is used in both wet-granulation and direct compression processes.

- It can be used as binder/diluent, lubricant and disintegrant which makes it suitable for tableting.
- Microcrystalline cellulose is also used in cosmetics and food products
• It is commercially available in different particle sizes and moisture grades that have different properties and applications.

Typical Properties

Melting point: 260–270°C.

Moisture content

Less than 5% w/w, however, amounts of water vary based on different grades. Microcrystalline cellulose is hygroscopic.

Particle size distribution

Mean particle size is 20 – 200 μm. Different grades may have a different nominal mean particle size

Solubility

It is slightly soluble in 5% w/v sodium hydroxide solution and insoluble in water.

Stability and Storage Conditions

Microcrystalline cellulose is a stable but it absorbs moisture. It is packed in a well-closed container in a dry and cool place.

Incompatibilities

Microcrystalline cellulose is incompatible with strong oxidizing agents.

Safety

It is nontoxic in nature and it is not absorbed by physiological membrane following oral administration hence it is not toxic. Consumption in large quantities of
cellulose may have a laxative effect. Intentional abuse of formulations containing cellulose, either by inhalation or by injection, has resulted in the formation of cellulose granulomas.

2.1.3 Colloidal silicon dioxide\textsuperscript{53}

Nonproprietary Names

BP: Colloidal anhydrous silica
PhEur: Silica colloidalisanzhdyrica
USPNF: Colloidal silicon dioxide

Synonyms

Light anhydrous silicic acid; silicon dioxide fumed; \textit{Cab-O-Sil M-5P}; silicic anhydride; fumed silica; colloidal silica;\textit{Aerosil}; \textit{Wacker HDK}; \textit{Cab-O-Sil}.

Chemical Name: - Silica

Molecular Weight: - 60.08

Structural Formula: - SiO$_2$

Functional Category

Viscosity-increasing agent; anticaking agent; suspending agent; tablet disintegrant; thermal stabilizer; Adsorbent; glidant emulsion stabilizer.

Description

Colloidal silicon dioxide is submicroscopic fumed silica with a particle size. It is a light, loose, bluish-white-colored, odorless, tasteless, non-gritty amorphous powder.

Specific gravity: 2.2

Stability and Storage Conditions

Colloidal silicon dioxide is hygroscopic but adsorbs large quantities of water without liquefying. It helps to increase the viscosity of system at a pH 0–7.5, colloidal
silicon dioxide is effective in increasing the viscosity of a system. However, viscosity-increasing properties increase at pH greater than 7.5 and decrease at a pH greater than 10.7. It is stored in a well-closed container. Few types of colloidal silicon dioxide have hydrophobic surface treatments, which reduces their hygroscopicity.

**Incompatibilities**

Incompatible with diethylstilbestrol preparations.

**Safety**

Colloidal silicon dioxide is widely used in oral and topical pharmaceutical products and it is generally regarded as safe. However, intraperitoneal and subcutaneous injection may produce local tissue reactions. So, it should not be used in parenteral preparations.

**Handling Precautions**

Hand gloves, mask and eye safety equipment must be used while handling. For larger quantities, a dustpirator is recommended.

**Regulatory Acceptance**

It is GRAS (Generally regarded as a safe) listed. It is enlisted in the FDA Inactive Ingredients Guide included in nonparenteral medicine in the UK.

**2.1.4 Polysorbate**

**Structural Formula:**
**Empirical Formula:** \((C_6H_9NO)_n\)

Polysorbates are fatty acid esters of sorbitol and its anhydrides copolymerized with a varying number of moles of ethylene oxide.

**Description:** Polysorbate 80 yellow colored, clear or almost clear, viscous liquid. It has a faint, characteristic odor; warm, somewhat bitter taste.

**Typical Properties:**

**Specific Gravity:** 1.07 to 1.09

**pH:** 6 to 8 (1:20 or 5% w/v aqueous solution)

**Hydroxyl Value:** 65-80

**HLB:** 15

**Viscosity:** 425 cP.

**Melting Point:** Softens at 150 °C.

**Flash point:** 113 °C

**Surface Tension:** 42.5 mN/m (0.1% w/v solution at 20 °C)

**Solubility:** Polysorbate 80 is very soluble in water, soluble in alcohol, cottonseed oil, corn oil, ethyl acetate, methanol or toluene. It is insoluble in mineral oil and liquid paraffin.

**Pharmaceutical Applications:**

These nonionic surfactants are very useful as emulsifying agents, forming O/W emulsions in pharmaceuticals, cosmetics and other types of products. It is used as solubilizing agent; wetting, dispersing / suspending agent; and as a pharmaceutical
aid (nonionic surfactant). It is used as solubiliser in oral and parenteral formulations and has shown enhanced dissolution of poorly soluble drugs from solid dosage form.

2.1.5 Talc

Synonym: Talcum

Structural formula:

![Structural formula](image)

**Molecular Formula:** Mg₆(Si₂O₅)₄(OH)₄

**Description:** It is a very fine, white to grayish-white, odorless, crystalline powder; impalpable, unctuous. It adheres readily to the skin and is soft to the touch and free from grittiness.

**Solubility:** Practically insoluble in water, dilute acids and alkalies, and organic solvents.

**Specific Gravity:** 2.7-2.8.

**Specific Surface Area:** 2.41-2.42 m²/g.

**Hardness (Mohs scale):** 1-1.5 depending upon the presence of impurities like calcium silicate and crystalline calcium carbonate.
Hygroscopicity: Talc absorbs significant amount of moisture at 25°C and relative humidity up to 90 %

Stability and storage condition: Talc is a stable material and may be sterilized by heating at 160 °C for not less than 1h.

Safety: Talc is not absorbed systemically and hence oral ingestion is nontoxic. However, intranasal and intravenous abuse of products containing talc can cause granulomas in the body tissues particularly in the lungs. Inhalation of talc causes irritation and prolonged exposure may cause pneumoconiosis. It has known to cause severe respiratory distress in infants. Talc contaminated with the asbestos has been concluded to be carcinogenic in the humans and asbestos free grade should therefore be used in the pharmaceutical products.

Uses: Pharmaceutical aid (dusting powder), either alone or with starch or boric acid; excipient and filler for pills, tablets and for dusting tablets molds; clarifying liquids by filtration. It is also used as anticaking agent, glidant, tablet and capsule diluent, lubricant. Talc was once widely used in oral solid dosage formulations as a lubricant and diluents. It is used as a lubricant in tablet and in an advanced powder coating for sustained-release pellets; and as an adsorbant. Talc is also used as a clarifying agent in the formulations.
2.1.6 Polyvinyl pyrrolidone (PVP)\textsuperscript{53}

**Synonyms:** Povidone, Polyvidone, PVP.

**Structural Formula:**

![Structural Formula](image)

**Molecular Formula:** $\text{(C}_6\text{H}_9\text{NO)}_n$

**Molecular Weight:** For PVP K-30 it is 50,000.

It is a synthetic polymer consisting of linear 1-vinyl-2-pyrrolidinone groups. The degree of polymerization decides molecular weights of polymer. It is produced commercially as a series of products having mean molecular weights ranging from about 10,000 to 700,000. The different types of povidone are characterized by their viscosity in solution, expressed as K-value, in the range 10 to 95.

**Description:** It is a fine, white to creamy white, odorless, hygroscopic powder. It has pH (1 in 20 or 5% w/v solution) in range of 3 to 7.

**Typical Properties:**

**Melting Point:** It softens at 150 \( ^\circ \text{C} \).

**Viscosity:** The dynamic viscosity of 5% w/v K-30 povidone solution in ethanol (95%) at 25 \( ^\circ \text{C} \) is 3.4mPa.

**Solubility:** It is soluble in water, alcohol, chloroform, and practically insoluble in ether, mineral oil.
Applications in pharmaceutical formulation

- Primarily used as binder in wet granulation process.
- It is used as disintegrant, dissolution aid and suspending agent.
- Povidone solution also used as coating agent.
- Also used as suspending, stabilizing or viscosity increasing agent in a number of topical and oral suspensions and solutions.

Chemicals/Solvents

Solvents, reagents and chemicals used for the study were of analytical grade.

List of equipments and chemicals used is provided in Table 4 and 5 respectively.

Table 4 : List of Equipments

<table>
<thead>
<tr>
<th>Instrument Name</th>
<th>Model</th>
<th>Make</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electronic Balance</td>
<td>AA-2200</td>
<td>Anamed</td>
</tr>
<tr>
<td>MagneticStirrer</td>
<td>2-MLH</td>
<td>Remi</td>
</tr>
<tr>
<td>Tablet compression machine</td>
<td>Rimek Mini Press-I, Alpha 1-2 LD plus</td>
<td>Karnavati</td>
</tr>
<tr>
<td>Freezedryer</td>
<td>Electrolab, TDT 08L</td>
<td>Electrolab</td>
</tr>
<tr>
<td>Dissolution test apparatus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV-Visible double beam Spectrophotometer</td>
<td>Sican 2301</td>
<td>Inkarp</td>
</tr>
<tr>
<td>FTIR</td>
<td>IRAffinity-1S</td>
<td>Shimadzu</td>
</tr>
<tr>
<td>Nanotrac</td>
<td>R- 150 Ultra</td>
<td>Microtrac</td>
</tr>
<tr>
<td>Differential Scanning Calorimeter</td>
<td>DSC-60</td>
<td>Shimadzu</td>
</tr>
<tr>
<td>Transmission Electron Microscopy</td>
<td>H-7500</td>
<td>Hitachi</td>
</tr>
<tr>
<td>pH Meter</td>
<td>EQ- 610</td>
<td>Equip-Tronics</td>
</tr>
<tr>
<td>Zetasizer</td>
<td>Nano S</td>
<td>Malvern</td>
</tr>
<tr>
<td>Disintegration test apparatus</td>
<td>-</td>
<td>Electrolab</td>
</tr>
<tr>
<td>Bulk density apparatus</td>
<td>-</td>
<td>Sakova</td>
</tr>
<tr>
<td>Hardness tester</td>
<td>-</td>
<td>Monsanto</td>
</tr>
<tr>
<td>ELISA Micro plate reader</td>
<td>-</td>
<td>Techno-scientific</td>
</tr>
<tr>
<td>Friabilator</td>
<td></td>
<td>Thermonik</td>
</tr>
<tr>
<td>Stability chamber</td>
<td></td>
<td>Lab control</td>
</tr>
<tr>
<td>Chemicals</td>
<td>Suppliers</td>
<td></td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Orlistat</td>
<td>Biocon Ltd, Banglore*</td>
<td></td>
</tr>
<tr>
<td>Capryol PGMC</td>
<td>Gattefosse, France*</td>
<td></td>
</tr>
<tr>
<td>Kollisolve PEG 300, Kollisolve PEG 400, Kolliphore EL, Kolliphore RH40</td>
<td>BASF India Limited, Mumbai*</td>
<td></td>
</tr>
<tr>
<td>Pancreatic lipase</td>
<td>Sigma Aldrich</td>
<td></td>
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<tr>
<td>Phenyl acetate</td>
<td>Fisher scientific</td>
<td></td>
</tr>
<tr>
<td>Acrysol EL-135, Acrysol K-150,</td>
<td>Corel Pharma Chem, Ahmedabad*</td>
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<tr>
<td>Folin Ciocalteu reagent</td>
<td>HimediaPvt Ltd. Mumbai</td>
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<tr>
<td>Triton X-100</td>
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<td></td>
</tr>
<tr>
<td>Chemicals</td>
<td>Central store of KLE University’s College of Pharmacy, Belgaum</td>
<td></td>
</tr>
</tbody>
</table>

*Samples were received as a gift samples for research purpose.
2.2 Methods

**Fig 4: Schematic presentation of workflow**

- **Preformulation study**
  - Identification Tests
    - Solubility studies
    - Melting point
    - FTIR analysis
  - Compatibility studies of drug
    - FTIR Analysis
    - DSC

- **Optimization study**
  - SEDDS
    - Saturation solubility
    - Identification of microemulsion zone
  - Liquisolid system
    - Determination of solubility
    - Determination of angle of slide
    - Determination of liquid retention potential

- **Characterization**
  - Surface Morphology
  - Differential scanning calorimetry
  - X-ray diffraction measurements
  - FTIR
  - Drug content
  - Droplet size analysis
  - Zeta potential
  - Preparation of compacts
  - Evaluation of SEDDS and LS Compacts
  - In vitro and in vivo evaluation
  - Stability studies
2.2.1 Preformulation study

Characterization of pure drug

UV spectroscopy (Determination of $\lambda_{\text{max}}$)\textsuperscript{34, 37}

A 10mg of Orlistat was accurately weighed and dissolved in 100ml of methanol and serial dilutions were carried out to obtain solution of 20µg / ml. The UV spectrum with scanning range of $\lambda_{\text{max}}$ 199-800nm was recorded using UV-spectrophotometer (Inkarp – Sican 2301).

Fourier Transform Infra-Red (FTIR) spectroscopy\textsuperscript{54}

The infrared spectrum of Orlistat was recorded over the wave number 4000 to 400 cm$^{-1}$ using FTIR spectrophotometer (Shimadzu).

Melting Point Determination

The melting point of orlistat was determined by capillary method. The melting point was further confirmed by DSC studies.

Solubility Determination\textsuperscript{55, 56, 57}

The shake flask method was used to determine the solubility of orlistat in various oils, surfactants and co-surfactants. An excess amount of drug added to each vial containing 5 ml of selected vehicle. These vials were vortexed manually and transferred in orbital shaking incubator (SAKOVA, scientific co.) at 72 h at 37 ± 1 °C. Further, samples were centrifuged at 10,000 rpm for 15 min. The supernatant obtained was diluted and filtered through cellulose acetate membrane filter (0.45 µm Himedia, Mumbai, India), and drug content was estimated by validated UV spectrophotometric method (Inkarp—Sican 2301).
2.2.2 Development of UV spectroscopic method for drug

Preparation of standard stock solution

The standard stock solution of Orlistat was prepared by dissolving 10 mg of drug in 25 ml methanol to obtain stock solution of 400 µg/ml concentration. It was filtered through Whatmann filter paper ≠ 41.

Preparation of sample solutions

The aliquots of stock solutions were subjected to serial dilutions to get solutions with concentration range of 8 – 28 µg/ml with phosphate buffer pH-8. Solutions were scanned in the range of 199 – 800 nm against blank and absorption maxima were found to be at 205 nm against blank and the calibration curve was plotted.

Validation of method

Linearity

The linearity was detected by testing different concentrations of the standard solution of Orlistat. Beer–Lambert’s concentration range was determined. The absorbance obtained at different concentration was plotted and further treated for linear regression analysis.

Precision

The precision of the method was studied in terms of intra-day changes in absorbance of drug solution (20 µg/ml) on the same day and inter-day changes on six different days over a period of one week. The Intra-day and Inter-day variations were calculated in terms of standard deviation.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were calculated by standard deviation of the response and slope of the calibration curve. The formulae for the LOD and LOQ are as follows
LOD = \frac{3.3 \sigma}{s} \quad \text{and} \quad \text{LOQ} = \frac{10\sigma}{s} \quad (5)

Where \( \sigma \) = Standard deviation of the response, \( S \) = Slope of the calibration curve

**Analysis of marketed capsule formulations**

The developed UV spectrophotometric method was used to determine Orlistat content into the commercially available marketed formulation of Orlistat (OrLean 60-Eris life sciences Pvt. Ltd.). Ten capsules were weighed and contents from capsule were obtained by removing capsule shell. The contents were mixed uniformly by using mortar and pestle. The quantity equivalent to 10 mg of Orlistat was weighed and transferred into the 25ml of volumetric flask containing 15ml of methanol, it was shaken for 15 min. Further, volume was made up with the methanol and it was subjected for sonication on bath sonicator for 10min. The solution was filtered through Whatmann filter paper # 41. This filtrate was diluted to get a solution of 20 \( \mu \)g / ml concentration. The absorbance was measured against blank solution. Orlistat content of capsule was calculated using the standard calibration curve. The drug content was ensured by performing HPLC analysis also.

**2.2.3 Drug-excipient compatibility study**

The drug was mixed uniformly (1:1) with other excipients like microcrystalline cellulose (MCC), plasdone F 29, sodium starch glycolate (SSG), oils and surfactant. It was stored at room temperature for one month. The mixtures of drug and excipients were then evaluated by using FTIR spectrometer (Shimadzu) and DSC (Shimadzu).
Differential scanning calorimetric study

A DSC-60 Differential Scanning Calorimeter (Make - Shimadzu) equipped with an intracooler and a refrigerated cooling system, which was used to analyze the thermal behavior of Orlistat with excipients mixture (MCC, Plasdone F29, SSG, Oils and Surfactant) in hermetically sealed flat aluminium crucibles, with temperature range from 0 to 100 ºC. The Blank crucible was used to calibrate the DSC temperature. Nitrogen was purged at 30 ml/min through cooling unit and peaks obtained were analyzed for drug excipient compatibility study.

FT-IR spectroscopic studies

Infrared spectra of Orlistat and mixture of drug and excipients (MCC, PVP, SSG, Oils and Surfactant) were obtained using Fourier-transform infrared (FTIR) spectroscopy (Shimadzu) by diffused cell technique. The spectra was recorded over the wave number 4000 to 400 cm⁻¹ and analyzed comparatively for drug excipient compatibility study.

2.2.4 Liquisolid drug delivery system

Preliminary study

Determination of Liquid load factor

It is defined as the maximum weight of liquid that can be retained by unit weight of powder material in order to get acceptable flowable powder admixture. To calculate, the required amount of the powder, flowable liquid-retention potentials (Φ - values) of Avicel PH102 and Aerosil 200 were determined. It is calculated by the following equation,

\[ L_f = \Phi + \Phi (1/R) \]
\[ \Phi = \text{Flowable liquid retention potential of carrier material} \]

\[ \Phi = \text{Flowable liquid retention potential of coating material} \]

Liquid load factor (Lf) was calculated by the following equation,

\[ Lf = \frac{W}{Q} \]  \( (7) \)

- \( W \) = Weight of liquid medication
- \( Q \) = Amount of carrier material

Optimum amount of the carrier powder was calculated by using following equation,

\[ R = \frac{Q}{q} \]  \( (8) \)

- \( q \) = Amount of coating material

### 2.2.5 Formulation of liquisolid tablets

Based on saturation solubility data non-volatile liquid vehicle with the maximum solubility was selected. Orlistat was dissolved into the non-volatile liquid vehicle (Capryol PGMC), allowed to mix by vortexing it manually, and if required, sonicated for 10 min. The required amount of liquid medication equivalent to a unit dose of 60mg was loaded on pre-mixed and pre-sieved calculated amount of the Avicel PH 102 and mixed thoroughly. Here, the alternative carrier material like NeusilinUS2® was tried to improve upon liquisolid approach\(^{15,58}\). Resulting powder mixture was blended with coating material Aerosil 200 in polybag to avoid particle size reduction, while preparing the blend two factors were taken into considerations i.e. excipients ratio (coating material: carrier material) and liquid load factor. Liquid load factor at three different levelviz 0.1406, 0.1800 and 0.2250 were considered whereas excipients ratio at 10, 15 and 20 were considered in accordance with preliminary trial data. In all batches, 4% and 1% of the Sodium starch glycolate and Plasdone F-29 were added. Finally, tablets were compressed at the strength of 60mg by using 10 station tablet compression machine (Rimek Mini Press-I, Karnavati) with
flat-faced punch and die size of 11 mm and 13 mm. The prepared powdered liquisolid tablets were evaluated for hardness and disintegration time.

2.2.6 Experimental design: $3^2$ factorial design

A $3^2$ factorial design was employed to formulate and optimize the LSDDS of orlistat. Two independent variables, excipients ratio and liquid load factor were determined at three different levels (low, medium and high) for their effect on dependent variables like, angle of repose and weight of tablet. Coded and actual levels for independent variables are indicated in Table 6. Optimized formulation with acceptable flow, lower tablet weight and maximum % drug release at 30min was selected for further study. All nine batches LS-1 to LS-9 prepared in triplicate. Data generated were subjected for the multiple regression analysis followed by 3-D response surface methodology in Stat graphics XVII to determine the effect of the excipients ratio (X1) and liquid load factor (X2) on the selected dependent variables. A statistical model incorporating interactive and polynomial terms was used to calculate the responses.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1 X_1 + \beta_{22} X_2 X_2$$

Where, $Y$ is the dependent variable, $\beta_0$ is the arithmetic mean response of the nine trials, and $\beta_i$ ($\beta_1, \beta_2, \ldots$) is the estimated coefficients for the corresponding factor $X_i$ ($X_1, X_2, \ldots$), which indicates the change in outcome when one factor is changed from low to high level. The polynomial terms ($X_1 X_1$ and $X_2 X_2$) were included to investigate nonlinearity.
Table 6: A $3^2$ Full Factorial Experimental design layout for LSDDS *

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Variable levels in coded form</th>
<th>Translation of Coded Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$X_1$</td>
<td>$X_2$</td>
</tr>
<tr>
<td>LS-1</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>LS-2</td>
<td>0</td>
<td>-1</td>
</tr>
<tr>
<td>LS-3</td>
<td>+1</td>
<td>-1</td>
</tr>
<tr>
<td>LS-4</td>
<td>-1</td>
<td>0</td>
</tr>
<tr>
<td>LS-5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LS-6</td>
<td>+1</td>
<td>0</td>
</tr>
<tr>
<td>LS-7</td>
<td>-1</td>
<td>+1</td>
</tr>
<tr>
<td>LS-8</td>
<td>0</td>
<td>+1</td>
</tr>
<tr>
<td>LS-9</td>
<td>+1</td>
<td>+1</td>
</tr>
</tbody>
</table>

*indicates -1 = low level, 0 = intermediate level +1 = high level.

2.2.7 Characterization of LSDDS

Angle of Repose

The angle of repose was determined using fixed-funnel free-standing cone method which was performed in triplicate for batches LS-1 to LS-9 by using the formula,

$$\theta = \tan^{-1}\left(\frac{H}{R}\right)$$  \hspace{1cm} (10)

Where, ‘$\theta$’ is angle of repose; ‘H’ is height between lower tip of the funnel and the base of heap of beads; and ‘R’ is radius of the base of heap formed.
Angle of slide\textsuperscript{61,62}

This is the specific evaluation for powder admixture used in liquisolid compact technique, to determine the flow properties of liquisolid admixture. The powder admixture to be tested is placed on one end of the metal plate (locally fabricated). Same end of the plate was raised until powder material slid completely. Angle is measured in between plate and ground surface.

**Carr’s Compressibility Index and Hausner’s Ratio**

The prepared powder blend of all batches (LS-1 to LS-9) were evaluated for flow ability in triplicate for Carr’s compressibility index (CCI) and Hausner’s ratio (HR) by using bulk density apparatus.

\[
CCl = \frac{(TD - BD)}{TD} \times 100 \tag{11}
\]

\[
HR = \left( \frac{TD}{BD} \right) \tag{12}
\]

Where, TD and BD are tapped density and bulk density respectively.

**2.2.8 Evaluation of LSDDS Tablet**

Compressed tablets were evaluated for the hardness, disintegration time and friability test. Test procedures were followed as per the procedures mentioned in the official books.
Content uniformity

Tablets were crushed and quantity equivalent to 10 mg of orlistat was weighed and transferred it into the 25ml of the volumetric flask containing 15 ml of methanol. It was kept for 15 min with frequent shaking. Further, volume was made up to 25 ml with methanol and it was subjected for sonication on bath sonicator for 10 min. The solution was filtered through Whatmann filter paper # 41. This filtrate was diluted with phosphate buffer pH 8 to get a solution of the 20 µg/ml concentration. The absorbance was measured on UV spectrophotometer (Inkarp – Sican 2301) against the blank solution.

2.2.9 Self-emulsifying drug delivery system (SEDDS)\textsuperscript{16}

Construction of ternary phase diagram\textsuperscript{56,63,64}

To optimize the concentration of the oil, surfactant and co-surfactant in SEDDS ternary phase diagram was plotted. By selecting SEDDS components at different proportions trial batches were prepared. 10 ml of each trial mixture was prepared and then allowed to mix by vortexing it manually in vial. A 50µL of the mixture was added in 50 ml of distilled water, followed by gentle stirring by magnetic stirrer. Finally globule size of the prepared emulsion was measured by using Nanotrac. Mixtures which were clear or slight turbid with minimum globule size were selected to draw emulsion region.

Formulation and process developmental study of SEDDS

Components used to prepare SEDDS were based on ternary phase diagram study. SEDDS. Castor oil, tween 80 and Capryol PGMC were added in different ratios in such a way that they will produce emulsions upon mixing with water by simple stirring with magnetic stirrer. While preparing SEDDS orlistat was added
mixed with Capryol PGMC and further Tween 80 and Castor oil was added to the system. Plasdone F-29 (0.2 % w/v) was used as a precipitation inhibitor. Prepared mixtures were subjected for homogenization to mix and dissolve all components uniformly. Globule size of prepared isotropic mixture was measured by diluting 50µL with 50 ml of distilled water.

2.2.10 Experimental design: 3² factorial design

A 3² factorial design was employed to formulate and optimize the liquid SEDDS of orlistat. Two Independent variables, quantity of oil, and ratio of surfactant to co-surfactant were tested at three levels for their effect on dependent variables like globule size and emulsification time. Coded and actual levels for independent factors are indicated in Table 7. Optimized formulation with minimum globule size was subjected for freeze drying. All nine batches SO-1 to SO-9 prepared in triplicate. Data obtained was subjected for the multiple regression analysis followed by 3-D response surface methodology in Stat graphics XVII to determine the effect of the amount of oil and amount of the surfactant, co-surfactant (S/Co-S) ratio on the selected dependent variables. Full factorial experimental design layout is shown in Table 3. A statistical model incorporating interactive and polynomial terms was used to calculate the response.

\[ Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 \ldots \ldots \ldots \ldots (13) \]

Where, \( Y \) is the dependent variable, \( \beta_0 \) is the arithmetic mean response of the nine trials, and calculated coefficients are \( \beta_i \) (\( \beta_1, \beta_2 \ldots \)) for the resultant factor \( X_i \) (\( X_1, X_2 \)), which indicates the change in outcome when one factor is changed from low to high level. The change in response is indicated by interaction term (\( X_1 X_2 \)). It gives idea
about how the changes occur if there is change in two factors at once. The polynomial terms ($X_1X_1$ and $X_2X_2$) are included to investigate nonlinearity.

**Table 7: A $3^2$ Full Factorial Experimental design layout for SEDDS**

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Variable levels in coded form</th>
<th>Translation of Coded Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$X_1$</td>
<td>$X_2$</td>
</tr>
<tr>
<td>SO-1</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>SO -2</td>
<td>0</td>
<td>-1</td>
</tr>
<tr>
<td>SO -3</td>
<td>+1</td>
<td>-1</td>
</tr>
<tr>
<td>SO -4</td>
<td>-1</td>
<td>0</td>
</tr>
<tr>
<td>SO -5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SO -6</td>
<td>+1</td>
<td>0</td>
</tr>
<tr>
<td>SO -7</td>
<td>-1</td>
<td>+1</td>
</tr>
<tr>
<td>SO -8</td>
<td>0</td>
<td>+1</td>
</tr>
<tr>
<td>SO -9</td>
<td>+1</td>
<td>+1</td>
</tr>
</tbody>
</table>

*indicates -1 = low level, 0 = intermediate level +1 = high level.

**2.2.11 Characterization of Self-emulsifying drug delivery system**

**Globule Size Measurement**

The particle size analyzer (Nanotrac R- 150 ULTRA, Microtrac Inc.) was used to determine the globule size and the samples were prepared by diluting 50 µL of SEDDS with 50ml of the distilled water in a beaker by using magnetic stirrer. The polydispersity index (PDI) was calculated to check the particle size distribution.

**Freeze thawing**

This test was performed to study thermal stability of formulations at different temperature conditions. One freeze thaw cycle consist of exposing formulation at -
20°C, 1-4°C and 40°C for 24h, 48h and 72h.

**Microscopic Observation of SEDDS**

The formulated SEDDS were observed through light microscope at 100 X power (Trinocular microscope, Metzer optical instrument Pvt. Ltd.) and images were taken for confirmation of their shape.

**Emulsification Time**

It is the time required to achieve homogenous mixture upon dilution. 1 g of the isotropic mixture was diluted with 0.1N HCl at 37ºc with gentle agitation using magnetic stirrer. Formulation was checked visually for the appearance.

**Centrifugation test**

Liquid SEDDS was diluted 1000 times with distilled water. Further, 2 ml of diluted SEDDS was filled in Eppendorf’s tube and centrifuged at 5000 rpm for 30 min, which was observed visually for phase separation.

**In vitro drug release study**

The *in vitro* drug release study was carried out by using USP Type II dissolution test apparatus (Electrolab). Dissolution media consisted of 3 % SLS and 0.5 % sodium chloride with pH 6.0 at a paddle speed of 75 rpm and temperature 37 ± 0.5 ºC. All the batches, SO-1 to SO-9, were evaluated for drug release property by dialysis technique. In this, one end of pre-soaked dialysis membrane (3.5 cm in length) was tied with thread, and isotropic mixture equivalent to 60 mg of orlistat was diluted with 2 ml of the dissolution medium was placed into dialysis bag. Dialysis tubing was tied at both ends with thread and allowed to rotate freely into the 900-ml dissolution medium. Aliquots of 5 ml were removed at different time intervals (10, 20, 30, 45, and 60 min) and diluted further with phosphate buffer of pH 8. Samples were analyzed by UV–visible spectrophotometer.
2.2.12 Preparation of the solid SEDDS by freeze drying method

The liquid SEDDS with minimum globule size was freeze dried to obtain Solid SEDDS. Freeze-drying was carried out by using Alpha 1-2 LD plus freeze dryer (CHRIST®) at a processing temperature of – 52 °C and vacuum 0.084 mbar. Lactose (5%) was used as cryoprotectant (Figure 5). Further self-emulsifying tablet was prepared by adding sufficient excipients to freeze dried product.

Fig 5: Freeze drying assembly used for preparation of solid SEDDS of orlistat.

2.2.13 Evaluation of LSDDS and SEDDS

Fourier Transform Infra-Red Spectroscopy (FTIR)

The procedure mentioned earlier was followed.

Powder X-Ray Diffraction Studies (PXRD)

The powder X-ray diffraction patterns of orlistat, LSDDS and SEDDS of orlistat were recorded by using Philips X-ray diffractometer and checked for polymorphic changes after formulation.
Differential Scanning Calorimeter (DSC-60)

The procedure mentioned earlier was followed.

Scanning Electron Microscopy (SEM)

The shape and surface analysis was performed by scanning electron microscope (Jeol, JSM 6360, Japan) at an original magnification of 500 X and the sample of raw orlistat and the liquisolid system were coated with gold in an argon atmosphere by an ion sputter coater (Jeol, JSM 1260, Japan). The sample was exposed for 120 seconds at current 20 mv.

In vitro drug release and dissolution profile comparison of LSDDS and SEDDS

Optimized LSDDS, SEDDS formulation and marketed formulation i.e. capsules (OrLean 60-Eris life sciences Pvt. Ltd.) were evaluated for the in vitro drug release in media with reduced concentration of the sodium lauryl sulphate. Dissolution media was consisted of 3, 2 and 1% Sodium lauryl sulphate (SLS) and 0.5% Sodium Chloride at a paddle speed of 75 rpm and temperature 37 °C ± 0.5 °C. Main intension was to screen suitable dissolution media, which shows the maximum drug release at minimal concentration of surfactant. In this, dissimilarity factor (f1) and similarity factor (f2) were calculated to compare dissolution profiles. Here, Similarity factor is one, which calculates the similarity in percent dissolution between the two curves. Similarity factor and dissimilarity factor were calculated by the following equation.70

\[
 f_1 = \frac{\sum R_t - \sum T_t}{\sum R_t} \times 100 \tag{14}
\]
\[ f_2 = 50 \times \log \left[ 1 + \left( \frac{1}{n} \right) \sum (R_t - T_t)^2 \right]^{0.5} \times 100 \]  

Whereas,

\( R_t \) = Dissolution rate of marketed formulation at time \( t \)

\( T_t \) = Dissolution rate of test product at time \( t \).

\( \geq 50 \) considered as the two products are equivalent.

### 2.2.14 In-vitro lipase inhibition study\(^{72,73,74,75}\)

#### Preparation of Substrate stock solution

Phenyl acetate (113 mg) was accurately weighed and dissolved into 10 ml of 0.1M Tris HCl buffer (pH 7) containing 1% Triton X-100. This solution was used for the preparation of aliquots with different concentration of substrate.

#### Preparation of Lipase solution

Pancreatic porcine lipase was selected as a model enzyme for study. An aqueous solution of an enzyme with concentration 5mg/ml was prepared and centrifuged at 10000 rpm for 10 min (Plasto crafts, industries (Pvt) Ltd, Mumbai-Superspin R-V/FA) to get a clear solution. The supernatant was used for the study. Sodium carbonate of 53 mM concentration was prepared by dissolving the 56.16mg of the sodium carbonate in 10 ml of distilled water.

#### Determination of linearity of the method

The linearity of measurements was evaluated by analyzing different concentrations of the substrate solution in the range of 20, 40, 60, 80,100\( \mu \)L whereas total volume was maintained at 210\( \mu \)L as shown in Table 8. To each tube 12 \( \mu \)L of the lipase solution was added. After incubation time of 20min 6 \( \mu \)L Folin Ciocalteu reagent was added. Absorbance of the prepared solutions was measured on ELISA micro plate reader (Techno-scientific). The calibration curve was obtained by plotting
the absorbance versus the concentration of substrate data followed by linear regression analysis.

Table 8: Showing the compositions of the aliquots with different substrate concentration.

<table>
<thead>
<tr>
<th>Amount of substrate (µL)</th>
<th>Amount of 53mM of sodium carbonate (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>190</td>
</tr>
<tr>
<td>40</td>
<td>170</td>
</tr>
<tr>
<td>60</td>
<td>150</td>
</tr>
<tr>
<td>80</td>
<td>130</td>
</tr>
<tr>
<td>100</td>
<td>110</td>
</tr>
</tbody>
</table>

Prepared liquisolid tablet, self-emulsifying tablet and marketed formulation were subjected for the dissolution linked lipase inhibition study to support the in vitro dissolution study data in terms of the in vivo lipase inhibition. The earlier reported colorimetric method for lipase activity was used with modification, which involves use of phenyl acetate as substrate. Principle of time dependent inhibition was based on the rate of hydrolysis of phenyl acetate by lipase enzyme which was inhibited by orlistat. Substrate was hydrolyzed by enzyme which liberates phenol and acetic acid which are soluble in water and does not form turbidity. Liberated phenol was detected by addition of Folin Ciocalteu reagent, which turns yellow color to blue color followed by absorbance measurement at 750nm. Measurement was carried out by using 96 well micro-plates (Tarsons, India) on ELISA micro-plate reader (Techno scientific). Phenyl acetate was used as substrate in 0.1M Tris HCL buffer pH 7
containing 1% of Triton X-100. Lipase suspension with 5mg/ml concentration was
used after centrifugation at 10000 rpm for 10mins. Test solution was prepared by
adding 148 µl of 53mM sodium carbonate, 12 µl of lipase suspension and 2µl of test
solution, which was allowed to incubate for 20min at 37ºc followed by addition of 60
µl phenyl acetate substrate solution further allowed to incubate for another 20mins.
Finally, 6 µl of the Folin Ciocalteu reagent was added to detect liberated phenol.
Positive control was prepared by adding 60 µl of substrate solution to the 148 µl of
53mM sodium carbonate solution. Test and Positive controls were estimated
simultaneously. All samples were estimated in triplicate followed by percentage
inhibition calculations.

\[
\text{% Activity of lipase} = \frac{\text{Avg. absorbance of test sample}}{\text{Avg.absorbance of positive control}} \times 100
\]  

(16)

\[
\text{% Inhibition} = 100 - \text{% Activity of lipase}
\]  

(17)

2.2.15 In vivo pharmacodynamic activity in Wistar rats

High-fat diet-induced antiobesity activity in Wistar rats

Male Wistar rats weighing about 150-200g were selected for the study. All rats
were housed in plastic cages in room with controlled temperature of 25 ± 2 ºC, 12:12
h light / dark cycle and having free access to water. Animals were divided in five
groups containing six animals in each group. All groups were served with normal
pellet chow. Animals were acclimatized for 1 week before starting the experiments.
Institutional animal ethical committee approval was obtained prior to the study. All
groups except normal group were fed with the high-fat diet (condensed milk 40 g +
bread 40 g) and (chocolate 15 g + biscuits 30 g + dried coconut 30 g). High-fat diet was provided for period of 40 days. The rats of the groups I was kept as normal control, whereas group II was treated as disease control. Groups III, IV and V were given 5 mg/kg Orlistat orally which was just given before feeding by oral gavage. Here, group III was treated with marketed orlistat formulation whereas group IV and V treated with self-emulsifying orlistat tablet and liquisolid tablet. Method of administration was same for marketed and developed formulation. The treatment was continued for 40 days. Test parameters like body weight and abdominal circumference were measured on day 1, then after each 10 days up to 40 days. Lipase activity was measured in all groups after 40 days. The statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test. P value (P < 0.05) was considered statistically significant.

Experimental design

Table 9: Shows grouping of animals

<table>
<thead>
<tr>
<th>Group</th>
<th>Details</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal</td>
<td>Water <em>ad libitum</em></td>
</tr>
<tr>
<td>II</td>
<td>Disease Control</td>
<td>High Fat (No Drug)</td>
</tr>
<tr>
<td>III</td>
<td>Marketed formulation</td>
<td>High Fat (Marketed orlistat formulation), Dose-20mg/kg orally</td>
</tr>
<tr>
<td>IV</td>
<td>SEDDS Tablet</td>
<td>High Fat + SEDDS Compact 20mg/kg orally</td>
</tr>
<tr>
<td>V</td>
<td>LSDDS Tablet</td>
<td>High Fat + LS Compact 20mg/kg orally</td>
</tr>
</tbody>
</table>

2.2.16 Histopathologic evaluation

Histopathology evaluation of animal in the group treated with the SEDDS formulation was carried to understand any mucosal damage occurred to the mucosal
lining of the stomach as this formulation contains surfactants. Isolated stomach tissue was kept in 10% neutral formalin solution, tissue were dehydrated, embedded in paraffin, cut into 5 μm section, stained with haematoxylin-eosin dye (H&E) stain and then observed for histopathological examination. Compound electron microscope was used.

2.2.17 Stability studies

Stability study was carried out at 40 °C/75% ± 5% RH for a period of three months by storing the liquisolid tablet and SEDDS tablet at stability chamber. The samples were withdrawn after 90 days and analyzed for drug content and in vitro release studies.

2.2.18 Statistical analysis

Data is expressed as mean ± standard deviation (SD). One way ANOVA followed by Dunnett’s test was used for the study (Graph pad prism 5, San Diego, CA, USA). A value of P < 0.05 was considered as statistically significant.