DOI: 10.56511/JIPBS.2016.v3.i04.pp17-25

e-ISSN: 2349-2759 p-ISSN: 2395- 1095

Journal of Innovations in Pharmaceutical and Biological Sciences (JIPBS)

www.jipbs.com



Research article

Reverse engineering and development of generic Orlistat formulation

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Key words: Orlistat, anti-obesity, weight loss, generic, differential solubility, reverse engineering.

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Abstract

Orlistat a weight loss drug is off patented in US in 2009, it has the annual sales of around $100 million of the innovator's product and has a high commercial potential in generic market. The marketed capsule formulation was decoded by differential solubility technique using methanol, methylene chloride and water as solvents and anti solvents. The quantity of API and excipients such as Polyvinyl pyrrolidone, microcrystalline cellulose, Sodium lauryl sulphate, sodium starch glycollate and talc obtained after decoding is 118.60, 7.85, 84.95, 6.80, 10.85 and 3.00 mg respectively. DSC thermogram of separated Orlistat gave a sharp endothermic peak at 46.5ºC. PXRD values of the separated API obtained at 2θ are 11.2, 12.2, 14.0, 15.4, 16.9, 18.3, 19.2, 22.5, 25.0. Based on the quantities of the API and excipients obtained after decoding, generic form of the Orlistat capsule was prepared, which consists of pellets in capsules. The prepared generic form was characterized for weight variation of ± 3%, angle of repose 28.5˚ and drug content of 95.35%. Generic dosage form had similar dissolution profile with that of marketed products and showed first order drug release mechanism with R^2 of 0.9591. Greater similarity factor for dissolution was established with a value of 70.13.

Introduction

The United States of America depends on a strong generic pharmaceutical industry to prevent the increasing cost of the prescription drugs; if this is not prevented then it affects national health care expenditures. As per the 'Department of Commerce' news there has been increased globalization in both brand-name and generic drugs over the last two decades. India, China, and Israel share the most manufacturing companies of generics, and the fastest-growing pharmaceutical manufacturing centers are in South Korea, Brazil, the Middle East, Russia, and Southeast Asia. The Department of Commerce reports that Australia, France, Greece, Japan, and Switzerland have generic-drug utilization rates below 30%, in contrast to the U.S. rate of 86%. Currently, Indian pharmaceutical companies account for 22 % of the generic world market. There is a tremendous growth in generic drug launches by Indian companies in America, which were 93 products in 2003 and over 250 products in 2008. This had been achieved only due to the reverse engineering technology [1, 2].

Decoding or estimating the qualitative and quantitative formula of the innovator's drug product popularly known as reverse engineering is a promising and most reliable technique in predevelopment of generic formulation, which is similar to innovators product. This technique gives the hidden content present in innovator's or reference formulation both qualitatively and quantitatively that has been masked by the innovator to prevent the entry of generic drugs into the market. After knowing the formula of the innovator formulation generic formulation of the same strength can developed or manufactured which is comparable to innovator's formulation in terms of quality, performance and intended use [3]. The data essential for the approval by the drug regulatory bodies to market a generic dosage form includes similarity of a dosage form with respect to physicochemical properties of an innovator product such as type of dosage form i.e tablet, capsule, syrup etc, quantity of API used its polymorphic form, quantity of the excipients present, *in-vitro* dissolution profile and bioequivalence studies are necessary. The *in-vitro* dissolution profile of a generic dosage form should have a similarity factor (F2) of above 50, which indicates that the generic dosage form will give comparable dissolution profile to that of the innovator's formulation, and finally succeed in bioequivalence studies [4].

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There are different stages of reverse engineering starting with the procurement of the innovators formulation determining the physicochemical parameters followed by decoding the quantitative formula, then solid-state characterisation of separated API and excipients and identifying the manufacturing process [2].

Orlistat chemically known as N-formyl-L-leucine (S)-1-[[(2S,3S)-3-hexyl-4-oxo oxetan-2-yl]methyl]dodecyl ester or tetra-hydrolipstatin used to treat obesity. Orlistat is off patented in U.S in year 2009, that's why this molecule has a big economic potential in present generic market. The study focuses on reverse engineering of Orlistat capsules formulation. Three marketed Orlistat capsule formulations were selected as reference product for decoding of formulations followed by its generic form development.

Experimental

Materials

The Orlistat was procured as a gift sample from Shreya Life Sciences Pvt ltd, Aurangabad, India. Sodium starch glycolate, sodium lauryl sulphate, microcrystalline cellulose, Polyvinyl pyrrolidone and talc were also obtained from Merck pharmaceuticals as a gift sample.

Methods

Characterization of procured API

The procured API Orlistat was analysed for its purity using different analytical techniques such as Fourier transform Infra Red spectrophotometer (FTIR), Differential scanning calorimetry Powder X-ray diffraction analysis, solubility and preliminary tests such as colour, odour & appearance [5].

Selection of wavelength and linearity

A standard stock solution of Orlistat was prepared by first dissolving accurately weighed quantity of orlistat and diluted suitably. The analytical wavelength selection was done by appropriate dilution of standard stock solution of 20 µg/ml and scanned separately in the range of 200-400nm in order to get good results. Aliquots of the stock solution were pipetted out from standard stock solution and diluted with dissolution media which comprises of 3% sodium lauryl sulphate, 0.5% Sodium Chloride and pH adjusted to 6 with the help of phosphoric acid to get the solutions of concentrations 10, 20, 30, 40, 50 µg/ml. The solutions were scanned at 212 nm in UV-visible spectrometer V-630 Jasco, Japan. Calibration curves were obtained by plotting the graph of absorbance versus concentration.

Fourier transform infrared analysis

The Infra-red spectra of solid dispersions were recorded by the potassium bromide (KBr) method using FTIR-V1700 Jasco spectrometer. Baseline adjustments were made using dried KBr and then spectrum of Pure Orlistat was recorded.

Differential scanning calorimetric analysis (DSC)

DSC analysis was performed to determine the purity of procured and separated Orlistat. The instrument used was Shimadzu TA 60 WS. Each sample was accurately weighed (1-3 mg) in an aluminium pan, crimped and hermetically sealed, while empty pan was used as reference. The system was calibrated with high purity sample of indium. The samples were scanned at a heating rate of 10˚C per minute. Over a temperature range of 20 to 130˚C under nitrogen atmosphere [6].

Powder X-ray diffraction study (PXRD)

Powder X ray diffraction pattern were measured in order to determine polymorphism or API purity of procured and separated drug. Measurements were performed using Philips X- ray generator PW 1830 equipped with copper anode (40 kV, 30mA) coupled to computer interfaced diffractometer control unit (XPERT-PRO). The scattered radiations were measured with vertical goniometer (PW 3050/60) [7].

Solubility of Orlistat and physical parameters

Solubility tests were performed in water, methanol and chloroform in accordance to their IP limits. Preliminary tests such as colour, appearance and odour were performed to characterise Orlistat powder.

Evaluation of marketed formulation of Orlistat

Preliminary physicochemical characterization of the marketed dosage forms were performed such as weight variation, assay, dissolution, disintegration, size of capsule, average particle size of capsule content and angle of repose[8].

Weight variation test

Twenty capsules were selected randomly and accurately weighted. The results are expressed in terms of ± SD. The test was performed to determine the percent deviation of each capsule weight with average weight. It passes the weight variation test only if no capsule deviates the range of ± 7.5 % of average weight.

Assay/drug content

Accurately weighed 100 mg powder of Orlistat was taken in a 100 ml volumetric flask containing 50 ml methanol. The powder was dissolved by shaking the flask and finally the volume was made up to 100ml. From this stock solution suitable dilutions of 10, 20, 30, 40 and 50 µg/ml concentration was prepared. These solutions were analysed in UV spectrophotometer and amount of Orlistat in capsule was determined with help of calibration curve [9].

Drug dissolution study

The in vitro drug release study was performed employing USP type I (Basket) Electrolab India was used, 900ml of dissolution media comprising of 3% sodium lauryl sulphate,

0.5% Sodium Chloride and pH adjusted to 6 with the help of phosphoric acid was prepared [10]. Temperature of the media was maintained between 36.5°C and 37.5°C, with stirring speed of 50 RPM. Aliqouts of 5 ml was withdrawn at time intervals of 10, 20, 30, 40, 60 and 90 minutes. The dissolution medium was replenished with same amount of fresh dissolution media at every withdrawal. The samples were filtered and solutions were scanned at 212 nm using UV-VIS spectrophotometer (V-630 Jasco,, Japan) and absorbance were recorded. Dissolution study was carried out on all three marketed formulations of Orlistat.

Disintegration test

The apparatus consists of a basket-rack assembly. The basket-rack assembly consists of six open-ended transparent tubes. One dosage unit and disc is placed in each of the six tubes of the basket. Water is used as the immersion fluid and temperature maintained at 35-39 °C. The requirements of the test are met if not less than 16 of the 18 dosage units tested are disintegrated.

Size of capsule and particle size of capsule content

The size of capsule was determined by measuring the quantity of sodium carbonate filled in the capsule shell. Out of the three two marketed formulations i.e Orlica and Obitrol consists of pellets and remaining one i.e. Zerofat was filled with powder. The suspension of Orlistat was prepared with the help of mineral oil. The suspended particles then placed on stage micrometer and average particle size was determined.

Angle of repose

Angle of repose was performed to determine the flowability of pellets. About 10gm of pellets were taken and allowed to pass through funnel, which leads to formation of hoop. The height and width of hoop was determined and with the help of formula $\theta = \tan^{-1} (h/r)$ angle of repose was determined, where θ is angle of repose, h is height and r is radius of hoop.

Decoding or separation of excipients

Step-I (Separation of API and Polyvinyl pyrrolidone)

The contents of 20 capsules were taken and powder equivalent to the weight of one capsule was taken and was treated with 30 ml of analytical grade methanol. Among the content of capsule only API and Polyvinyl pyrrolidone are soluble in methanol. The methanolic solution of capsule content was filtered with the help of Whatmann filter paper. The residue was kept aside and filtrate was allowed getting air-dried. The entire methanol was evaporated in about 30 minutes. After the evaporation of methanol, the dried powder is then dissolved in 15 ml of distilled water to separate polyvinyl pyrrolidone from API (Orlistat) which is practically insoluble in water. After separation of API and Polyvinyl, pyrrolidone both the powders are air dried and their quantities are accurately measured.

Step-II (Separation of microcrystalline cellulose)

Microcrystalline cellulose is freely soluble in methylene chloride. The dried residue obtained after treatment of capsule content with methanol is then treated with methylene chloride. The residue was dissolved in 15 ml of methylene chloride and filtered through Whatmann filter paper. At this moment the filtrate consists of only microcrystalline cellulose as it is soluble in methylene chloride and the residue is insoluble which consists of remaining excipients. The filtrate is allowed to get air dried for 30 minutes. After air drying, the quantity of separated microcrystalline cellulose is determined.

Step-III (Separation of SSG and SLS)

Sodium starch glycolate (SSG) is soluble in chilled water, whereas sodium lauryl sulphate (SLS) is soluble in warm water. The residue obtained after treatment with methylene chloride is treated with chilled water. Sodium starch glycolate get separated. The remaining residue, which is soluble in warm water, is sodium lauryl sulphate. With the help of procedure described above all the excipients except talc are separated and their quantity per capsule is determined. By extracting the quantities of separated content of capsule from average weight of content of per capsule gives the amount of talc present per capsule.

Characterization of separated API and excipients

The separated excipients are further dried to remove traces of moisture and other volatile impurities present. The melting point of each excipient was determined using digital melting point apparatus. FTIR spectra of each excipient was analysed and interpreted. The characteristic peak of each excipient was studied and compared with pure excipient. The refractive index of each separated excipient was also determined to ensure the purity of the separated excipients. The refractive index was measured with the help of Abbes refractometer.

Preparation of generic form of Orlistat

Based upon the results obtained by decoding of marketed capsule formulation containing pellets the quantities of the separated excipients were confirmed. The next step was to develop generic formulation i.e. similar to the marketed formulation of Orlistat. At this step the quantities of excipients and API are known but the process and steps involved in the preparation of pellets of Orlistat is yet to be ascertain, therefore preliminary studies was performed which are as follows; the pellets of microcrystalline cellulose (MCC) were prepared by conventional process of extrusion. The possibility of pelletization and appearance of pellets was observed. The consistency of damp mass, pellet's appearance and pellet's strength were studied. The pellets are prepared using extruder (model SELEC-RC 100A, marketed by Anish Pharma equipment Ltd; Nasik). Before preparation of pellets the extruder was calibrated for revolution per minute (RPM) to ensure the correctness of

speed of extruder. The sieve no #44 was used for preparation of pellets. The excipients used were same as by the innovator product and the quantities of excipients were weighed as per the decoded quantities of the innovator product. The quantity of API and each excipient is mentioned in Table no 1. All the excipients and API are accurately weighed and passed through sieve no. 40 to remove solid agglomerates if present and then thoroughly mixed except talcum using tumbling mixer for about 30 minutes. The damp mass was prepared by using water quantity enough to obtain dough like consistency. Damp mass was transferred to the hopper of extruder. The extruder was set to 25 RPM, extruder was allowed to revolve for 20 minutes, and the pellets were collected in tray. The obtained pellets were weighed on digital balance and the yield was recorded. The pellets were allowed to air dry for 30 minutes and yield of dry pellets was recorded. The dried pellets were filled in capsules of size zero. Table no. 1 states the quantities of API and excipients.

Characterization of prepared generic form of Orlistat

The characterization of prepared generic form included determination of Weight variation test, Assay, Dissolution, drug release kinetics, average particle size, angle of repose. The procedure for each characterization test was already had been discussed.

Weight variation test

Twenty capsules were selected randomly and accurately weighted. The results are expressed in terms of ± SD. The test was performed to determine the percent deviation of each capsule weight with average weight. It passes the weight variation test only if no capsule crosses the range of 90 to 110% of average weight.

Assay/drug Content

Accurately weighed pellets equivalent to 200 mg drug were crushed in a dried mortar- pestle. Powder of the pellets was dissolved in up to 100 ml phosphate buffer pH 7.4. It was stirred for 15 min and filtered. Appropriate dilutions of solution were prepared subsequently from it and were analyzed by UV-VIS spectrophotometer (UV-1700, Pharmaspec, Shimadzu Ltd, Japan) at 275 nm.

Particle size analysis

Particle size of prepared extrudates was measured by optical microscope (Olympus CX31, Japan).

Friability

Accurately weighed quantity of extrudates (3 gm) were taken from final batch of pellets and placed in a friabilator and tumbled for 100 revolutions at 25 RPM. Twelve steel balls (weighing 0.445 gm each) were used as an attrition agent. Subsequently, the pellets were sieved through sieve no. 20. The weight loss (%) is calculated as:

$$\% F = (Wi-Wr/Wi)\ 100$$

Where, Wi is initial weight of pellets before friability testing, and Wr is the weight of pellets retained above the sieve after friability testing.

Drug dissolution study

The in vitro drug release study was performed employing USP type I (Basket) Electrolab India and drug release was estimated by similar procedure as that of the marketed formulations described earlier.

Drug release kinetics

The dissolution profile of all the formulations were fitted to zero order kinetics, first order kinetics, Higuchi, Hixson-Crowell, Korsmeyer and Peppas to ascertain the kinetic modeling of drug release by using a PCP Disso Version 2.08 software, and the model with the higher correlation coefficient was considered to be the best model[11-14].

DSC, PXRD and solubility study

Differential scanning calorimetry and Powder X ray diffraction analysis was performed as per the method described in characterization of API section.

Determination of similarity factor (F2)

The similarity factor (F2) is a logarithmic transformation of the sum-squared error of differences between the test T_t and reference products R_t over all time points .It represents the closeness of two comparative formulations. F2 ranges approximately from 0 to 100. The SUPAC-IR suggested that

Table 1. Physical parameters of separated API and excipients

Sr. no	Ingredient	Quantity obtained (mg)	Melting point		Refractive index	
			Observed (˚C)	Reported (˚C)	Theoretical	Practical
1.	Orlistat	118.60±0.8	46.6±0.2	46.0	1.46	1.46±0.25
2.	SSG	10.85±0.3	202±0.4	200	1.46	1.46±0.30
3.	MCC	84.95±2.5	261±0.3	260-270	1.50	1.51±0.12
4.	PVP	7.85±0.13	152±2.2	150-155	1.42	1.41±0.16
5.	SLS	6.80±0.11	201±1.6	204	1.46	1.46±0.28
6.	Talc	3.00±0.05	153±1.5	152.4	4.59	4.58±0.19

similarity between two dissolution profiles be concluded if F2 is between 50 and 100. Generally similarity factor in the range of 50-100 is acceptable according to US FDA. It is calculated by using the following formula proposed by proposed by Moore and Flanner [15].

$f_2=50 \log \{[1+1n\sum n-1n (Rt-Tt) 2] - 0.5*100\}$

Results and Discussion

Characterization of procured API

Selection of wavelength and linearity of Orlistat

The analytical wavelength selected was 212 nm after scanning in the range of 200-400nm. Linearity of Orlistat after plotting concentration versus absorbance using concentration range of 10 to 50 µg/ml obtained was R^2 = 0.999.

Fourier transform Infrared analysis of Orlistat

The Infra-red spectra of solid dispersions of Orlistat (API) showed different peaks enlisted below which confirms the compound to be Orlistat; A peak at wave number 3450-3400 cm^{-1} due to NH stretching. A peak at 1720-1705 cm^{-1} was obtained indicating the presence of C=O stretching. The two prominent peaks at 2950-2850 and 1690-1630 cm^{-1} is due to C-H stretching of alkyl group and C=O stretching of amide group respectively.

Differential scanning calorimetric analysis (DSC)

The DSC thermogram of procured Orlistat was obtained which shows that the melting starts at 40˚C and gave a sharp endothermic peak at 46.6˚C. Therefore orlistat was characterized by a melting peak at 46°C. The sharp change in peak is due to endothermic change in the substance. The heat flow down of procured Orlistat was found to be 242.2 mW as shown in figure 1.

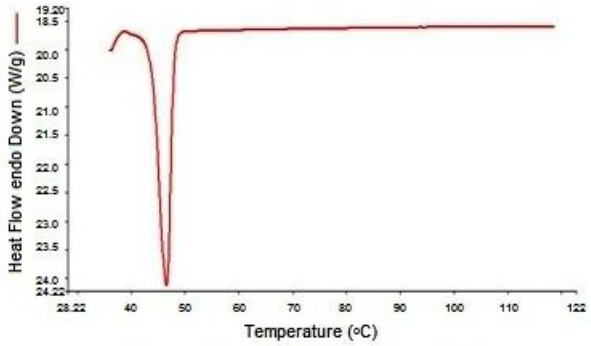


Figure 1. DSC thermogram of procured Orlistat

Powder X ray diffraction study (PXRD)

The XRD spectrums of procured API were studied for the characteristic peaks. Both XRD spectrum shows peak at same 2θ value. The spectrum show intense peak at 2θ value 9.0, 9.3, 9.8, 10.0, 10.8, 11.2, 12.2, 14.0, 15.4, 16.9, 18.3, 19.2, 22.5, 25.0. The similarity in peak indicates purity and same crystal form of procured Orlistat as shown in figure 2.

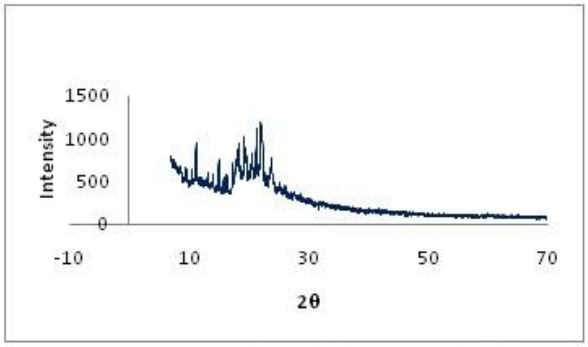


Figure 2. PXRD of procured API

Solubility of Orlistat and physical parameters

Orlistat was found to be practically insoluble in water, but freely soluble in methanol, and soluble in other organic solvent. The procured Orlistat was found to be white crystalline powder and without any specific odour.

Evaluation of marketed formulation of Orlistat

Weight variation test

Weight variation test was performed on 20 capsules on three reference product separately. The average weight and weight variation of Orlica, Obitrol and Zerofat capsule formulation observed was 242.10 ± 5.14, 240.66 ± 1.57 and 240.18 ± 1.35 respectively.

Assay/drug content

The assay was performed using methanol as solvent and 20 µgm per ml as stock solution. Compare the absorbance of dilution of sample with calibration curve of standard bulk sample. The absorbance is studied at 212 nm. The % purity of Orlica, Obitrol and Zerofat capsule was found to be 94.44%, 91.45%, 90.69% respectively.

Drug dissolution study

The dissolution studies of three reference product were carried out for 0 to 90 minutes. All the three reference products gave approximately similar dissolution profile and percent drug release obtained was above 90% after 2 hours of dissolution. The results obtained were plotted as time (in minutes) vs percent drug release as shown in figure 3.

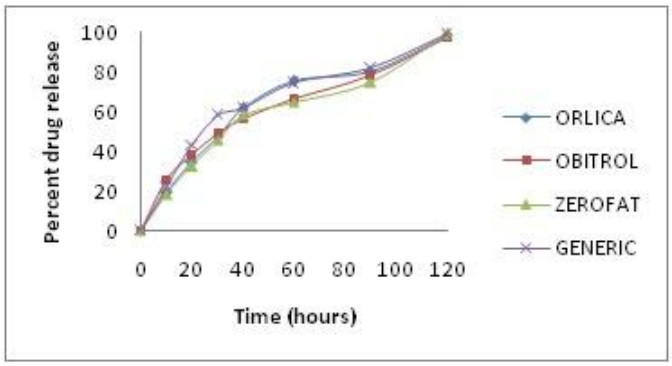


Figure 3. Drug release profile of marketed and generic (prepared) Orlistat dosage forms

Disintegration test

The disintegration time of Orlistat capsule was determined by using water as disintegration media at temperature of 37˚C. The disintegration time for Orlica, Obitrol and Zerofat capsule was found to be 22 minutes, 24 minute and 25 minutes respectively.

Size of capsule and particle size of capsule content

The size of capsule was determined by filling the sodium bicarbonate in the empty capsule. The amount of powder which was filled in the capsule was found to be 0.68 gram. Thus the size of capsule is size 0. Orlica and Obitrol contain pellets while Zerofat contain powder form. The average size of pellets in Orlica and Obitrol is 1430 and 1361 µ. And the average particle size of powder in Zerofat capsule is 456µ. Average particle size of content of capsule was determined with the help of optical microscopy. Determination of least count-It was determined by observing division of stage micrometer coinciding with eyepiece. Least count = 6/5 = 1.2.

Angle of repose

When determined with the help of formula $\theta = \tan^{-1} (h/r)$ the angle of repose for Orlica, Obitrol and Zerofat was found to be 24.64˚, 25.39˚, 28.82˚ respectively.

Decoding or separation of excipients

Step-I (Separation of API and Polyvinyl pyrrolidone)

Excipients are separated on differential solubility basis; API and Polyvinyl pyrrolidone both are soluble in methanol. Hence in the first step both these constituents were separated using methanol. Then Methanolic fraction was allowed to evaporate and the solid obtained was treated with water, since Polyvinyl pyrrolidone is soluble in water and Orlistat is practically insoluble in water. The water was removed with the help of distillation and traces of water were removed in a dryer. The quantity of API obtained was 118.60 mg and the amount of Polyvinyl pyrrolidone obtained was 7.85 mg.

Step-II (Separation of microcrystalline cellulose)

The residue obtained after treatment with methanol was then treated with Methylene Chloride in which only microcrystalline cellulose is freely soluble, which is then separated in crystalline form by evaporating methylene chloride. The amount of microcrystalline cellulose is found to be 84.95 mg.

Step-III (Separation of SSG and SLS)

Sodium lauryl sulphate is freely soluble in warm water, which can be easily separated by dissolving in water. The amount of Sodium lauryl sulphate was found to be 6.80 mg per capsule. Sodium starch glycolate is soluble in chilled water only and hence chilled water of temperature about 4˚C is used to separate the sodium starch glycolate from talc which is insoluble in water. The amount of sodium starch glycolate was found to be 10.85 mg. All the excipients are separated except the talc. Thus, remaining amount of solids is nothing but talc. Thus, the amount of talc is 3.00 mg.

Characterization of separated API and excipients

Fourier transform Infrared analysis of separated API and excipients

The Infra-red spectra of separated API and excipients showed different peaks enlisted below which confirms the compound to be Orlistat. Characteristic peaks at 3450-3400 cm^{-1} showing NH stretching. Peaks at 1720-1705 cm^{-1} exhibiting C=O stretching of ketone. The C-H Stretching of Alkyl shows peaks at 2950-2850 cm^{-1}. The C=O stretching of Amide gave the peaks at 1690-1630 cm^{-1}. The FTIR spectra of Orlistat is shown in figure 4.

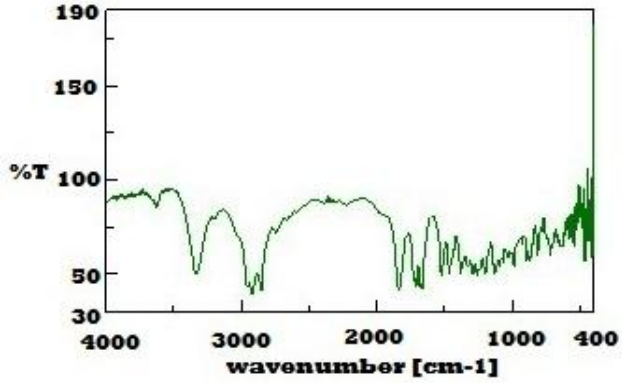


Figure 4. FTIR spectra of separated API

Sodium lauryl sulphate

A broad peak at wave number 3500 cm^{-1} was observed due to presence of OH group. A peak at 2900 cm^{-1} was obtained indicating the presence of aromatic group. Two peaks were observed at 1248 and 1216 cm^{-1} is due to S-O stretch. The FTIR spectra of the Sodium lauryl sulphate are shown in figure 5.

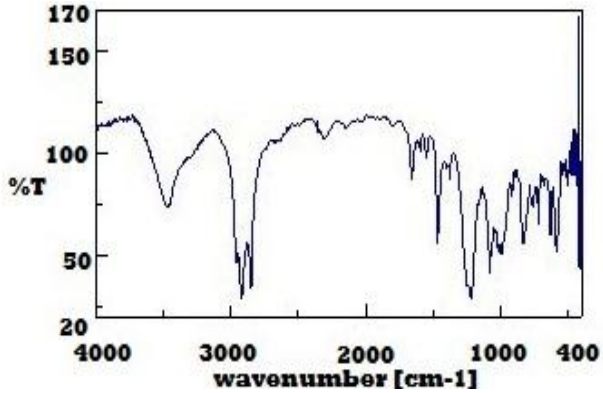


Figure 5. FTIR spectra of sodium lauryl sulphate

Talcum

A strong peak at 3677cm^{-1} and less intense peak at 3660cm^{1} which shows the OH stretching and vibration of $Mg(OH)_2$ and Mg_2FeOH groups respectively. The FTIR spectra of talcum is depicted in figure 6.

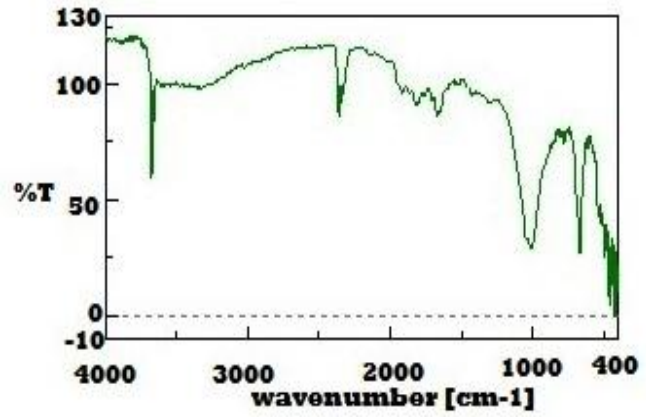


Figure 6. FTIR spectra of talc

Sodium starch glycolate

A broad peak at 3500 cm^{-1} is due to OH group. An intense peak at wave number 2900 cm^{-1} is due to aromatic group and peak at 1600 cm^{-1} is due to ketone group. The FTIR spectra of the Sodium starch glycollate is shown in figure 7.

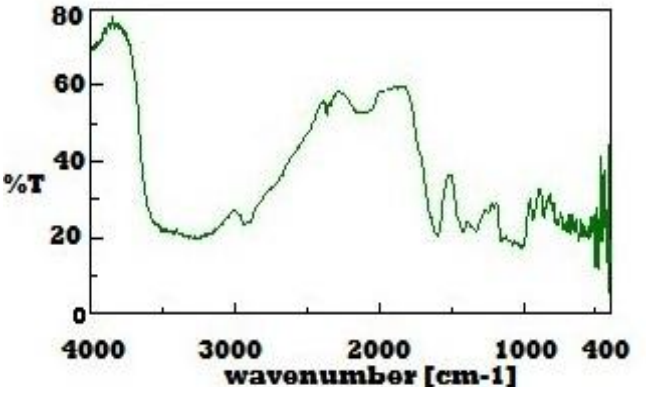


Figure 7. FTIR spectra of Sodium starch glycolate

Polyvinyl pyrrolidone

A sharp peak was observed at 2900 cm^{-1} which shows CH stretching. A peak at 1900 cm^{-1} confirms ketone group. The FTIR spectra of the Polyvinyl pyrrolidone is shown in figure 8.

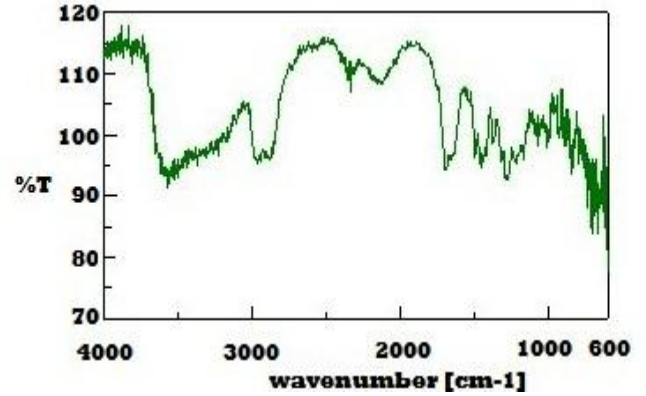


Figure 8. FTIR spectra of Polyvinyl pyrrolidone

Determination of refractive index of separated excipients

The refractive index of each excipient and Orlistat was determined with the help of Abbe Refractometer. The results are shown in Table no 1.

Preparation of generic form of Orlistat

Calibration of extruder

The extruder was set to 25 RPM and revolutions were counter checked with the help of digital counter in set of triplicate. The RPM of extruder was found to be 25.

Development process

The damp mass of the API and all the excipients were prepared. For 15 gm of powder mixture 8 ml of water was required. Kneading was done for 10 minutes and the mass was transferred in extruder for preparation of pellets. The RPM of extruder was set at 25 RPM. The weight of wet pellets was 6.6 gm. The final weight of dry pellets was 6.4 gm. The LOD of dried pellets was 0.2%. The dried pellets were mixed with 3.00 mg of talcum as a lubricant. Finally the prepared pellets were filled in size 0 capsule manually.

Characterization of prepared generic form of Orlistat capsule

The generic form is then characterised with the help of various physical and chemical tests such as weight variation, average particle size, dissolution, disintegration etc.

Weight variation test

Weight variation test was performed on 20 prepared capsules. The weight variation was found to be ± 4%. As no capsule deviates the range of ± 7.5 % of average weight, hence the prepared capsules pass the test.

Assay/drug content

The drug content of generic drug was 95.35%, which is comparable to the reference dosage form. The % purity of Orlica, Obitrol and Zerofat was found to be 94.44%, 91.45%, 90.69% respectively. The % purity range indicates the similarity between prepared dosage form and reference dosage form.

Drug dissolution study

Drug dissolution study of all the tree reference products and generic drug product gave approximately same dissolution profiles with complete drug dissolution in 120 minutes. The dissolution results are depicted in figure 6.

Drug Release kinetics

All the three marketed as well as prepared generic form of the Orlistat formulation showed first order drug release mechanism, as the plots of *in vitro* release profiles of all formulations showed highest linearity i.e R^2 = 0.9478 for

Zerofat, 0.9539 for Obitrol, 0.9532 for Orlica and 0.9591 for prepared generic form of Orlistat as shown in Table 2.

Average particle size of prepared pellets

The average size of manufactured pellets was found to be 1482 µ when determined with the help of optical microscopy method.

Angle of repose

The angle of repose for prepared pellets was found to be 28.5˚. The corresponding angle of repose indicates the good flow property of prepared pellets.

Differential scanning calorimetric analysis

The differential scanning calorimetric analysis (DSC) thermogram of separated Orlistat was obtained which shows that the melting starts at 40ºC and gave a sharp endothermic peak at 46.5ºC which is similar to DSC thermogram of procured API depicted in figure 7. Therefore orlistat was characterized by a melting peak at 46°C. The heat flow down of procured Orlistat was found to be 242.2 mW as shown in figure 9.

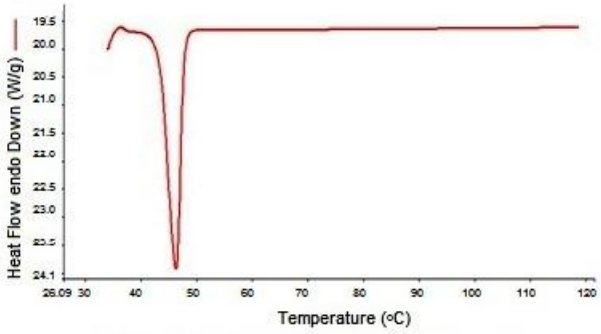


Figure 9. DSC thermogram of separated Orlistat

Powder X ray diffraction study (PXRD)

The PXRD spectrums of separated API were studied for the characteristic peaks. Both XRD spectrum shows peak at same 2θ value. The spectrum show intense peak at 2θ value 9.0, 9.3, 9.8, 10.0, 10.8, 11.2, 12.2, 14.0, 15.4, 16.9, 18.3, 19.2, 22.5, 25.0. The similarity in peak indicates purity and same crystal form of procured Orlistat as shown in figure 2 and 10.

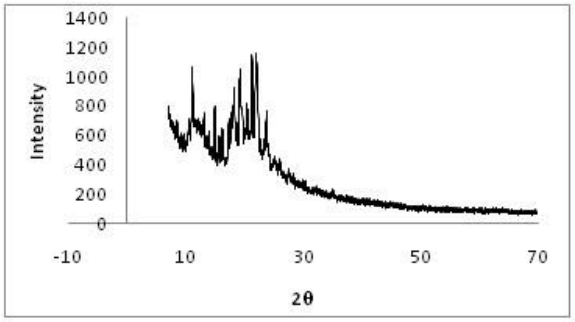


Figure 10. PXRD of separated API

Determination of similarity factor (F2)

The similarity may be compared by model independent or model-dependent methods e.g. by statistical multivariate comparison of the parameters of the Weibull function or the percentage dissolved at different time points, or by calculating a similarity factor e.g. the F2 similarity factor. With the help of dissolution data the F2 value for manufactured capsule was determined. The dissolution study was carried out from 0 to 90 minutes and percent drug release was determined. When F2 value was determined, it was found to be 70.13. The generic product to be bioequivalent with the innovator product the F2 value should be above 50 and thus the prepared generic form of Orlistat is having similar dissolution profile of marketed formulation of Orlistat with less than 10% deviation.

Table 2. Release kinetics of prepared and marketed formulations of Orlistat

Release kinetics	Zerofat (R^2)	Obitrol (R^2)	Orlica (R^2)	Prepared pellets (R^2)
Zero order	0.8842	0.8786	0.8825	0.8812
First order	0.9478	0.9539	0.9532	0.9591
Higuchi	0.8842	0.8786	0.8825	0.8794
Korsmeyerpeppas	0.9421	0.9442	0.9433	0.9488
Hixson crowell	0.8427	0.8659	0.9248	0.9126

R^2 - Correlation coefficients

Conclusion

The reverse engineering of marketed formulation of Orlistat Capsule was successfully performed, which was confirmed by conducting characterization of active and inactive ingredients present in the innovator's product by different analytical techniques. Based on the qualitative and quantitative data obtained by de-formulation of marketed Orlistat Capsule, generic orlistat capsules were prepared. The similarity factor i.e. F2 was found to be 70.13, which indicates that the prepared generic Orlistat capsule is similar to the innovator's product and can be further explored for the bioequivalence testing to be approved by the regulatory bodies.

Acknowledgement

It is an honor for us to thank honorable madam Mrs. Fatma Rafiq Zakaria, Chairperson Maulana Azad Education Trust for providing necessary facilities for the research work. We

would also like to thank Shreya Life Sciences Pvt ltd and Merck pharmaceuticals for providing gift samples.

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