ORIGINAL ARTICLE

Formulation Development and Validation of a Method for Direct, Underivatized Analysis of Orlistat in Orlistat-Loaded Solid Dispersion in Simulated Conditions

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Abstract

There is a high need of enhancing solubility of poorly soluble orlistat drug and its underivatized analysis in dosage forms due to high excipient interference present in the formulations. In this research, we 1st time account an easy, fast, selective, accurate, and specific UV–visible spectrophotometric technique and preparation of solid dispersion (SD) with cooperative effect of poloxamer 188 and spray-dried lactose. SDs were prepared by kneading method and in developed analytical technique, drug recognition was carried out using UV–visible spectrophotometer at λ max of 217.5 nm using methanol as solvent. The process validated for various parameters according to the present International Conference on Harmonization guidelines. The method demonstrated good recoveries for samples fortified with three different concentration ranges of low, mid, and high level covering the working range. The calibration graph was correlating in the concentration range of 1–10 µg/ml with the correlation coefficient of 0.9993. The accuracy was found to be in between 99.3 and 100.9%. The precision among six samples preparations was within limit, limit of detection and limit of quantification values are 0.07 and 0.238 µg/ml, respectively. The percentage recovery of the drug was found to be 100.2% which indicates that there was no interference of the capsule or other excipients with the method. The enhanced solubility of prepared SDs was translated to improved dissolution of the drug when compared with crystalline and amorphous form complementing the outcome of the solution state study. Dramatically high improvements in the dissolution rate of orlistat were achieved by a special supportive effect of poloxamer 188 with spray-dried lactose.

Key words: Orlistat, UV–visible spectrophotometer, International Conference on Harmonization guidelines, validation, solid dispersions

INTRODUCTION

Obesity is very common problem with young generation, as well as old people due to lifestyle changes and junk food. To treat this problem nowadays, people uses a drug known as orlistat. Orlistat usually acts by blocking the lipase thus reducing the absorption of fat that you eat or keeping it from being absorbed by our body. This medicine reduced the total calorie intake from the diet. It is mainly used with consultation from a health provider to reduce body fat. Xenical and Alli are some marketed drugs available for the orlistat as OTC drug in some of the countries.

Orlistat is a saturated derivative of lipstatin, as shown in Figure 1, which a strong innate inhibitor for pancreatic lipases which was obtained from the bacterium *Streptomyces toxytricini*. It was chosen over lipstatin for obesity treatment due to its quality and safety.[1]

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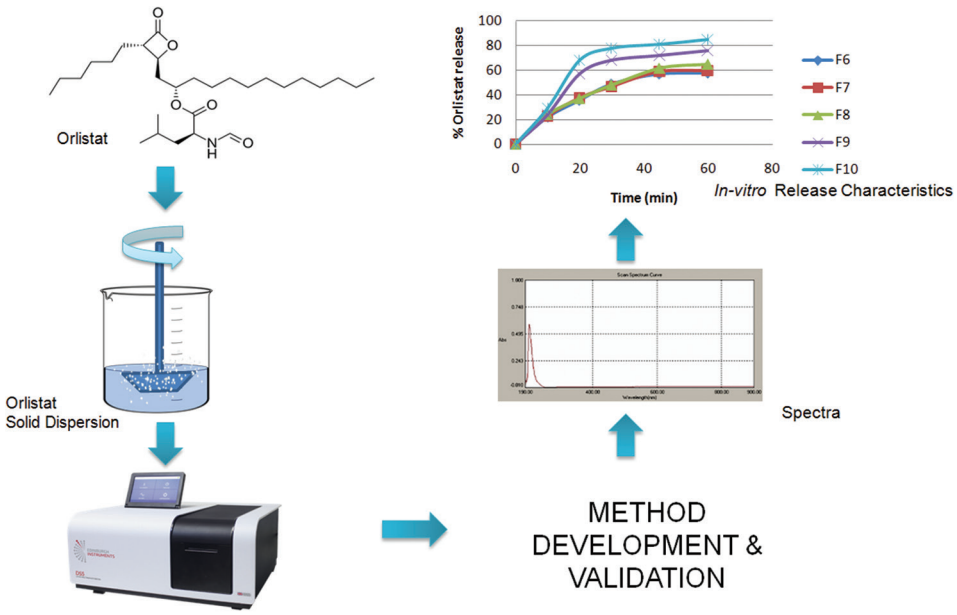
GRAPHICAL ABSTRACT



Development and validation of UV spectroscopic method for the estimation of orlistat from solid dispersions and its application in *in vitro* dissolution study

Orlistat acts by blocking the gastric and pancreatic lipase, these are the enzymes which break down the fat present in the intestine into triglycerides. When the function of these enzymes is blocked, then triglycerides present in the food are not able to get converted into fatty acids and thus get removed from the body without being absorbed through feces.[2]

Thioesterase area of fatty acids synthase was recently found to be block by the orlistat drug. These enzymes were found to help in proliferation on cancer cells but do not affect the regular cells of the one body.[3] The probable adverse effects of orlistat are like blocking of the cellular off-targets or low bioavailability. One study depicted the chemical proteomics method which can be utilized to find latest cellular target of orlistat together with other drug targets.[4]

Orlistat was normally taken as the dose of strength 120 mg 3 times in a day previous to the meals as per the standard prescription of the drug. It reduces approximately around 30% of intake dietary fat from being absorbed by the body.[5] Orlistat is a BCS II class category, means it is poorly water-soluble drug but has good permeability due to which it is having limited dissolution activity and less activity. Hence, its action on the lipase can be improved by reducing the particle size or by improving its saturation solubility. The dissolution of orlistat can be estimated by very well-known Noyes Whitney Equation (i)

$$\frac{dC}{dt} = \frac{SD(Cs - Ct)}{L}$$

Where, dC/dt is the dissolution rate, S is the surface area of the particles of the drug, D is the diffusion coefficient, L is the diffusion layer, Cs is the saturation solubility, and Ct is the drug concentration at time t.

Various analytical methods have been reported using HPLC, LC–MS, UPLC, and other techniques for analysis of orlistat in plasma and urine but all are quite expensive and complicated. Hence, many formulations are available in market as single drug and in combination with other drugs but none showed prominent increase in the solubility of the drug. All these factors call for the requirement for a formulation with high solubility and a method which is simple, easy, fast, responsive, accurate, specific, and reliable for the estimation of orlistat in pharmaceutical preparations as well as in bulk drug. The main objective of the current effort is to prepare an orlistat formulation with high solubility and a process for the routine investigation of orlistat by UV–visible spectrophotometer, which is quiet easy to perform in routine analysis. The proposed also method decreases the analysis time for the drug while avoiding any interference from the excipients or other ingredients of the formulation.[6]

MATERIALS AND METHODS

Materials

Orlistat was provided as a gift sample from CMG Biotech, India. Methanol, ethanol, acetone, chloroform, and acetonitrile AR Grade used were of CDH Chemicals, India. Poloxamer 188, hydroxyl propyl methyl cellulose (HPMC), mannitol, and spray-dried lactose were procured from Merck Chemicals, India. Marketed formulation (A) was purchased

from the local market and all other supplementary materials used in the study are of analytical quality grade.

Preparation of Orlistat polymer films

HPMC was selected as polymer for making films due to its hydrophilic nature and excellent film forming ability. Films were prepared by dissolving orlistat and HPMC in a combination of ethanol: water (80:20 v/v) on a magnetic stirrer and the solvent was evaporated in the vacuum oven at about 40°C for the time period of around 4 h. To choose the optimum composition of orlistat and HPMC [Table 1], different formulations were prepared with HPMC concentration ranging from 14% to 23% of the total weight. The prepared films were powdered in a glass mortar pestle to prepare powder then mannitol was added to make it free flowing. The obtained sample containing about 120 mg of orlistat was filled in capsules. The prepared formulations were analyzed using differential scanning calorimetry (DSC), Fourier-transform infrared spectroscopy, and dissolution testing for drug release.

Preparation of orlistat solid dispersion (SD) by kneading method

Poloxamer 188 was selected as polymer for SD due to its surfactant activity. Orlistat and poloxamer 188 mixture in different ratios [Table 1] were wetted with incorporation of little quantity of ethanol and kneaded properly for about 30 min in mortar and pestle to make smooth dough. The prepared dough was passed through sieve no. 120 to make granules and then desiccated in vacuum about 12 h. The obtained dried granules were then reduced into powder mass in a glass mortar and pestle and mixed with spray-dried lactose to get a free flowing mass. The powder mass containing about 120 mg of orlistat was filled into capsules of size 3 and analyzed for drug release through dissolution testing.

Analytical method development of orlistat

Instrumentation

Double-beam UV–visible spectrophotometer model (Kyoto, Japan) 1601 with 10 mm cell length and quartz cells were used for the analytical purpose and method development.

Selection and optimization of solvent

Solvents have a very profound effect on the quality and the sharpness of the peak. Various solvents such as methanol, chloroform, acetone, and water were used to get the best peak in a particular solvent. All solvents were optimized and, out of them, methanol was found to give satisfactory results relating to quality and shape of the peak. Methanol also showed no interference from any other components at the provided wavelength.[7]

Standard stock solution

The orlistat stock solution with a concentration of 10 µg/ml was prepared in methanol. The various dilutions were made by taking 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 ml from the stock solution and then make up the volume up to 10 ml from methanol to get the different dilutions of the drug from 1 to 10 µg/ml.

Selection of wavelength

The wavelength which showed the maximum absorption in the spectral scan was selected as the wavelength for the further analysis of the samples. 217.5 nm was found to be the best for the scanning of the drug samples.

Method validation

International Conference on Harmonization (ICH) guidelines were used for the validation and development of the method. All parameters were evaluated for the proposed method.

Sensitivity

Limit of detection (LOD) and limit of quantification (LOQ) were determined for the estimation of sensitivity of the method. Different concentrations of the orlistat solutions were utilized for the estimation of LOD and LOQ.

The LOD can be estimated by Equation (ii) as:

$$LOD = 3.3\ \sigma/S \quad \text{(ii)}$$

The limit of quantitation (LOQ) can be estimated by Equation (iii) as:

Table 1: Formulations of orlistat with HPMC and poloxamer 188

Formulation composition	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Orlistat (mg)	120	120	120	120	120	120	120	120	120	120
HPMC (mg)	30	35	40	45	50					
Poloxamer 188 (mg)						40	45	50	55	60
Ethanol: water (80:20) (ml)	5	5	5	5	5	5	5	5	5	5
SD Lactose (mg)						60	55	50	45	40
Mannitol (mg)	70	65	60	55	50					
Total weight (mg)	220	220	220	220	220	220	220	220	220	220

LOQ = 10 σ/S (iii)

Where, σ = standard deviation

S = slope of the standard curve.

The slope was obtained from the equation of the calibration curve.

Specificity and selectivity

Orlistat different marketed formulations were used for the assessment for the specificity of the technique. The dilutions of the orlistat of concentration 5 µg/ml were made in methanol and of the marketed formulation were analyzed by the developed method. The marketed formulation estimated amount was compared with the orlistat standard amount.[8]

Linearity and range

Least square regression method was used for the estimation of the linearity of the standard curve. Ten different concentrations of orlistat were prepared of the range from 1 to 10 µg/ml utilizing methanol as the medium.

The method of least square was used to find the association among the concentration of orlistat (x) and the observed absorbance (y). To understand this concept, let's take the experimental value points be X_i and Y_i.

If we assume that $Y_i = \alpha+\beta X_i+e_i$, the e_i represents residuals and a and b are the values for intercept and the slope of the graph can be represented by the Equations (iv) and (v) as given.

$$b = \frac{n\sum_{i=1}^{n} x_i y_i - \sum_{i=1}^{n} x_i \sum_{i=1}^{n} y_i}{n\sum_{i=1}^{n} x_i^2 - [\sum_{i=1}^{n} x_i]^2} \quad \text{(iv)}$$

$$a = \frac{\sum_{i=1}^{n} y_i \sum_{i=1}^{n} x_i^2 - \sum_{i=1}^{n} x_i \sum_{i=1}^{n} x_i y_i}{\sum_{i=1}^{n} x_i^2 - [\sum_{i=1}^{n} x_i]^2} \quad \text{(v)}$$

Standard deviation of slope (S_b) can be calculated by the equation as given by Equation (vi):

$$S_b = \sqrt{\frac{\sum_{i=1}^{n} (y_i - y_p)^2}{(n-2)}} * \sqrt{\frac{1}{\sum_{i=1}^{n} (x_i - x_p)^2}} \quad \text{(vi)}$$

Where, x_p and y_b are the arithmetic mean value of x_i and y_i

Standard deviation of intercept (S_a) can be calculated using the Equation (vii)

$$S_a = \sqrt{\frac{\sum_{i=1}^{n} (y_i - y_p)^2}{(n-2)}} * \sqrt{\frac{1}{\sum_{i=1}^{n} (x_i - x_p)^2}} * \sqrt{\frac{\sum_{i=1}^{n} x_i^2}{n}} \quad \text{(vii)}$$

Correlation coefficient (r)

The value of "r" can be obtained by applying the Equation (viii)

$$r = \frac{[\sum_{i=1}^{n} (x_i - x_p)(y_i - y_b)] / (n-1)}{[\sum_{i=1}^{n} (x_i - x_p)^2 (y_i - y_b)^2] / (n-1)^2} \quad \text{(viii)}$$

By application of all these equations, one can easily and accurately estimate the values for linearity and selected the appropriate values for the calibration range.

Accuracy

Accuracy of the technique was estimated by utilizing standard analysis method at three different concentration levels. Different concentrations of standard drug were prepared as 2.5, 5, and 7.5 µg/ml from the stock solution and their estimation was done by the proposed method. To the above prepared solutions 0.5, 1, and 1.5 µg/ml, orlistat was added to pre-analyzed solutions and again analyzed in the same way to estimate the total amount of the drug.[9]

Precision

For the estimation of the precision of the technique, the repeatability and reproducibility were done using three concentration levels, that is, 2.5, 5, and 7.5 µg/ml using methanol as the solvent. Repeatability was done on 3 different days and reproducibility was done using two different UV–Visible spectrophotometers. The statistical term mostly used to represent precision was standard deviation, which can be obtained by applying Equation (ix)

$$\sigma = \sqrt{\frac{\sum (x_i - \mu)2}{N}} \quad \text{(ix)}$$

Where, σ = sample standard deviation

N= the size of the sample.

Relative standard deviation (RSD) or coefficient of variance can be obtained by applying Equation (x)

%RSD = (σ/µ)*100 (x)

Robustness

The method was evaluated for the robustness by doing small changes in the method such as wavelength, instrument, operator, and checking the percentage effect on the proposed method.

Orlistat content test in SD

The weighted powder samples were dissolved in 25 ml of a combination (distil water to ethanol, 1:1, v/v) for the purpose

of determining the concentration of orlistat in SD after mechanical treatment, respectively. All SD components were fully dissolved and then evaluated in all cases.

DSC and powder X-ray diffraction

Thermal analysis and X-ray diffraction analysis of samples were carried out using DSC-550 instrument and DRON-4 equipment.

Scanning electron microscopy

The Hitachi TM-1000 microscope was used to collect electronical pictures (Tokyo, Japan). Gold was used to coat samples using a fine auto coater JEOL JFC-1600 (Tokyo, Japan). The laminate parameters were 30 s, 30 mA, and 15 nm film thickness.

Solubility determination

Excess samples were dissolved in distilled water with shaking in the orbital shaker (200 rpm) for about 12 h at 37°C to test solubility. Finally, samples were filtered and analyzed.

Dissolution media

To perform dissolution test, USP II paddle types apparatus was used with dissolution medium consisting of 3 % SLS (sodium lauryl sulfate) in 0.5 % NaCl at the pH of around 6.0 ± 0.5 at the paddle speed of about 75 rpm. The temperature of the dissolution media was kept at 37°C and one capsule in each vessel. The samples were withdrawn (aliquot of 5 ml) at the pre-determined intervals of 0, 10, 20, 30, 45, and 60 min.

Application of the proposed method for prepared SD and marketed dosage forms

Assay of the prepared and marketed formulation

The amount of orlistat in capsules (labeled claim: 120 mg) was measured by powdering the contents of 20 capsules and weighing the powder corresponding to 10 mg of orlistat.[10] Methanol was used to extract the medication from the powder. Then, it was sonicated for about 30 min to dissolve the drug completely, and the amount was made up to 100 ml. At 2500 rpm, the resultant solution was centrifuged for around 10 min and supernatant estimated for the concentration of the drug.

Relevance of the proposed method for the dissolution data examination

The prepared formulations samples subjected to dissolution testing were estimated by planned analytical method. All the obtained samples were collected and filtered with the help of a membrane filter (0.45 µm) and then analyzed for estimating concentration of orlistat.

Stability studies of SDs

The stability study of SDs was carried at temperatures of 35 ± 2°C and under stress conditions of 45 ± 2°C and 60 ± 2°C for a period of 3 months according to ICH Q1. The samples were taken periodically to analyze the drug content of SDs capsules.

Analysis of release mechanism

In vitro release kinetics of drug from SDs capsules was analyzed by mathematical modeling. The obtained data were applied to various release kinetics models such as zero order, first order, Higuchi model, and Hixson-Crowell model. The suitable release model was selected based on values of correlation coefficient, release constant, and diffusion exponent obtained from curve fitting of release data.

RESULTS AND DISCUSSION

The analytical technique used come out to be easy, responsive, precise, specific, inexpensive, and fast for the regular evaluation of orlistat formulations and active pharmaceutical ingredient.

Estimation of the drug

Orlistat melting point comes out to be 41°C and the solubility of drug in water was almost in negligible amount. It was found to be freely soluble in ethanol, methanol, and acetone. In the spectral scan λmax of orlistat was found around 217.5 nm. Whereas other methods[11] report the estimation of orlistat by indirect method, where the drug was combined with a reagent to make it colorful and then detect it in the visible range.

Parameters for the method validation

The analytical technique was validated as per the ICH guidelines Q2 (R1).

Linearity and range

The linearity of the proposed technique reflects the measurement of the amount of the correlation among the response and the concentration of the drug. It was performed by assessing a single measurement at multiple analyte concentration and then processing the data using linear least square regression analysis. Acceptable linear correlation was found among the absorbance and the concentration of orlistat in the chosen range of 1–10 µg/ml. The various typical parameters were found to be 0.021 ± 0.16 for slope and 0.008 ± 0.19 for the intercept. The correlation coefficient was found to be 0.9993 and the regression coefficient was around 0.9992 [Table 2] among the orlistat drug concentration and the mean absorbance of the analyte.

Precision

Precision of the developed technique represents the nearness of degree of scatter among a series of recovered data through several sampling of the similar standardized sample underneath the approved circumstances. The intraday precision was done to verify the application of analytical process within the same laboratory under a small interval of time utilizing the similar operator conditions with the same apparatus while the interday precision involves determination of the effect of slight variation in analytical method when the same procedure was utilized in a laboratory on dissimilar days by some other technician. Repeatability was find out by estimating same three dissimilar concentrations (2.5, 5.0, and 7.5 µg/ml), 3 times a day and intermediate precision was estimated by finding out the same three different concentrations (2.5, 5.0, and 7.5 µg/ml), 3 times a day for a minimum of 3 different days [Table 3].

The standard deviation, percent RSD, and confidence interval for the intra-assay precision, intermediate precision, and reproducibility for all the three different concentration levels were come out to be under 0.017, 0.367, ±0.016 and 0.018, 0.638, ± 0.012 and 0.10, 100.011, and ±0.084, correspondingly. The value obtained here indicates an outstanding intraday precision, intermediate precision, and reproducibility of the developed technique.

Table 2: Outcome of validation parameters found by the developed method

Validation parameters	Result obtained
λ max	217.5 nm
Beer's law range (µg ml^{-1})	1-10
Slope±SD	0.021±0.16
Intercept±SD	0.008±0.19
Correlation coefficient	0.9993
Accuracy	99.1–101.4%
Precision (%RSD)	0.39
LOD (µg ml^{-1})	0.07
LOQ (µg ml^{-1})	0.238

Table 3: Intra- and inter-precision studies (*n*=3)

Amount of drug injected (µg/ml)	Amount of drug detected (µg, mean±SD)	%RSD
Intraday (*n*=5)		
2.5	2.48 (±0.012)	0.492
5.0	5.00 (±0.016)	0.350
7.5	7.49 (±0.013)	0.235
Intraday (*n*=5)		
2.5	2.49 (±0.012)	0.434
5.0	5.00 (±0.014)	0.398
7.5	7.49 (±0.016)	0.289

Accuracy

Accuracy of the developed technique is defined as the nearness of measured value to the true value. Normally, accuracy was represented and calculated by recovery studies through the appliance of analytical method for the estimation of analyte by standard addition practice. In this practice, the standard amount of analyte was spiked with pre-analyzed samples solutions.[12,13]

The difference between the sample result with or without added constituent provides information regarding the amount of recovery from the added constituent.[14] The % recovery for all the three concentration levels was found to be range from 99.5% to 101.7% with confidence interval range of 0.093–±0.210. The outcome received out of the standard addition and reference analysis methods were also establishing the support for the accuracy of the developed technique.[15]

Specificity

The existence of excipients in the dosage forms was not found to obstruct with the obtained analyte peak. Hence, the developed technique was established as precise and selective for analyte drug estimation.

LOD/LOQ

LOD and LOQ were found to be:

LOD=3.3 σ/S =0.07µg/ml

LOQ=10 σ/S = 0.238µg/ml

Robustness

The developed method was found to be adequately robust, as the deviation in the λ max was found in the limits of ± 1.0 nm with % recovery coming out in the range of 99.2–99.8 with a greatest % confidence interval of ±0.008.

Physical characterization studies of orlistat SDs

The DSC thermograms and X-ray diffractograms of free orlistat, physical mixtures (PMs), and orlistat SD with electron micrographs are shown in Figure 2.

Relevance of the developed UV–visible spectrophotometer technique on the marketed formulation

Assay values

As per the ICH guidelines, the assay value of all the formulations comes out to be in the range of 99.96–100.21

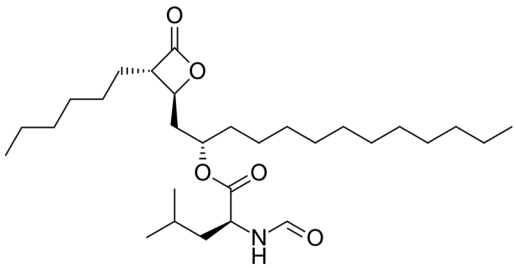


Figure 1: Structural formula for Orlistat

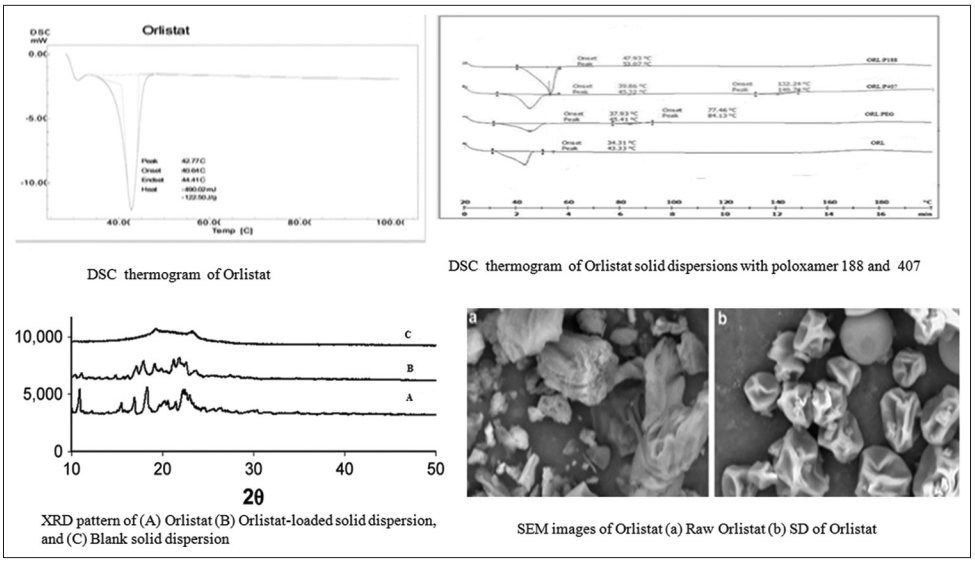


Figure 2: Physical characterization studies of orlistat SD by DSC, XRD, and scanning electron microscopy

with utmost % confidence interval of ± 0.16 [Table 3], indicating the selectivity of the assay method.

Drug release from the formulations

The proposed technique was useful for the evaluation of orlistat in the marketed tablet formulations and prepared formulations and there release pattern is shown in Figures 3 and 4. The marketed formulation (A) showed about 35% of drug release whereas formulation F5 showed about 55% drug release but the best formulation was found to F10 which showed maximum release of drug about 85%. Thus, it can be concluded that surfactant poloxamer 188 increases the solubility of orlistat. As poloxamer has amphiphilic property, it helps in solubilization and stabilization of lipophilic compounds.[16] It has low toxicity compared to other surfactants and self-assembly property that can be leveraged to encapsulate drug using an array of different processing techniques.

Stability studies

Optimized formulation F10 was loaded for the evaluation and formulation of stability experiments at 35 ± 2°C, 45 ± 2°C, and 60 ± 2°C, as shown in Table 4, there was no significant change in the percentage of drug content and pH of the formulation. The results predict regarding safe drug polymer miscibility and solubility[17] with no physical instability of amorphous drug and drug-polymer interactions. Storage conditions are not significantly affecting the thermodynamic and kinetic stability of amorphous SDs.

Release pattern

The release of the medication from the formulations is important, especially in modified release and fast

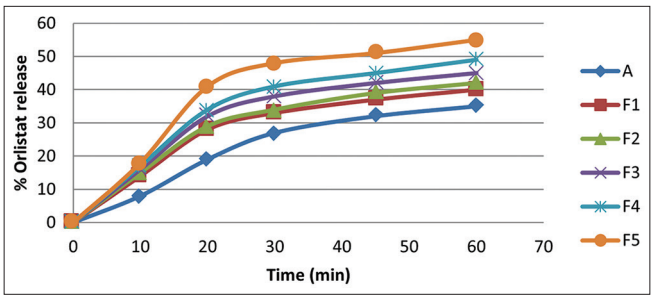


Figure 3: Result of the ratio of orlistat/HPMC on the *in vitro* release characteristics of the prepared SD in comparison with the marketed formulation (a)

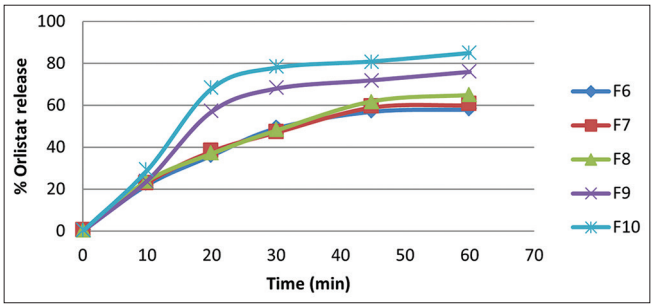


Figure 4: Result of the ratio of orlistat/poloxamer 188 on the *in vitro* release characteristics of the prepared SD

release dosage forms. Several factors, including drug physicochemical qualities, excipients, dosage form design, manufacturing process variables, and dosage form design, influence drug release from dosage form. It is critical to investigate the medication release pattern from the dosage form since it influences the dosage form's effectiveness.[18] Several models aid in understanding the release pattern, allowing for the development of an effective formulation. Drug release of the selected dosage forms [Figure 5] when analyzed by different release kinetics models such as zero

Table 4: Evaluation data of formulation subjected to stability studies

Formulation code	Month 1		Month 2		Month 3	
	Drug content ± SD	pH ± SD	Drug content ± SD	pH ± SD	Drug content ± SD	pH ± SD
Stability study at 35 ± 2°C/65% RH	97.04%±1.23	6.11 ± 0.63	95.04%±0.87	6.11 ± 0.45	93.02%±1.84	6.31 ± 0.34
Stability study at 45 ± 2°C/70% RH	93.91%±0.98	6.13 ± 0.43	93.21%±1.83	6.12 ± 0.72	90.93%±1.24	6.15 ± 0.81
Stability study at 60 ± 2°C/75% RH	92.78%±1.72	6.32 ± 0.68	91.78%±1.49	6.17 ± 0.51	89.90%±1.63	6.31 ± 0.62

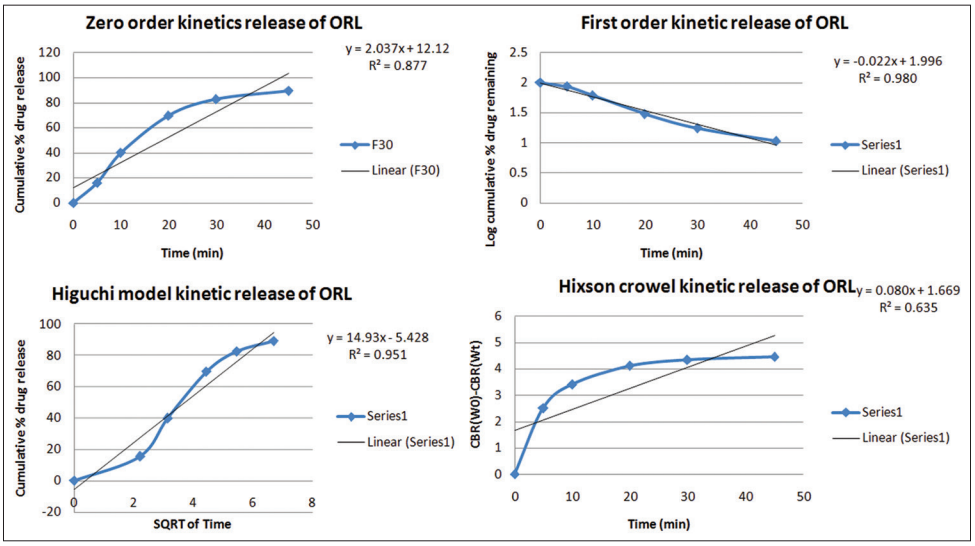


Figure 5: Different release kinetics of selected formulation (F10)

order, first order, Higuchi model, and Hixson-Crowell model shown first-order release kinetics based on values of correlation coefficient (as the value of R^2 was found to be highest), release constant, and diffusion exponent obtained from curve fitting of release data.

CONCLUSION

The prepared SDs showed enhanced solubility and improved dissolution of the drug when compared with crystalline form and marketed formulations complementing the outcome of the study. The elevated level of the dissolution rate of orlistat was achieved by compliment effect of poloxamer 188 with spray-dried lactose. Validated analytical technique was developed for the estimation of orlistat by UV–visible spectrophotometer. The method was easy, consistent, precise, and reproducible. It avoids the lengthy extraction process thus decreases the testing time and possibility of practical errors. Small cost, fast estimation time, acceptable precision, better specificity, and the ability to evaluate clearly the drug in the existence of excipients present in the pharmaceutical formulations. As per the ICH guidelines, the developed technique was validated successfully and simplicity of the technique can be suitably utilized for the regular analysis of orlistat in prepared SDs, API, marketed tablets, and other formulations. The method was equivalent and better compare to the accessible method in all respect and also to the methods which examine the analyte in the plasma.

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