

Stability Indicating Stress Degradation Study of Orlistat by UV Spectrophotometry Method

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ABSTRACT

Objectives: To study the stress degradation behavior of an orlistat by UV spectrophotometric method. **Methods:** An accurate, simple and rapid UV spectrophotometric method for orlistat was developed and used in phosphate buffer pH 8. The method is validated for linearity, precision and accuracy. Stress degradation study was performed at alkaline, acid, neutral and photolytic conditions. **Results:** Linearity was found to be 8-28µg/ml. Absorbance showed at different concentrations was found to be linear with the correlation coefficient 0.999. The Intra-day and Inter-day precision studies were carried out. The relative standard deviation was found to be 0.0029 and 0.0095 for Intra-day and Inter-day precision studies respectively. Orlistat was found to be degraded in acidic, alkaline, neutral and oxidative stress conditions whereas it was found to be stable in light. The LOD and LOQ of the developed method were found to be 0.3444µg/ml and 1.0740µg/ml respectively. Accuracy was studied by the recovery method. Percentage accuracy was found to be 96.19% ± 0.99%. The decrease in absorbance was observed at 1h, which concludes the degradation of the drug in acidic stress conditions. The drug was found to be unstable in alkaline conditions.

The results revealed that degradation products are having more absorbance than the drug. The 5 is slight decrease in absorbance was observed at 1h, which remained constant after 4 h. also method showed its usefulness in analyzing marketed samples of orlistat formulations. **Conclusion:** From the obtained results, it can be concluded that the developed method is simple and economic. It can be successfully used for the stress degradation studies; hence, it can be applied for routine analysis in the industry.

Key words: Orlistat, Stress degradation, UV spectrophotometry.

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INTRODUCTION

Orlistat is widely used as an antiobesity agent. Chemically it is [(1S)-1-[(2S, 3S)-3-hexyl-4-oxo-oxetan-2-yl] methyl] dodecyl] (2S)-2-formamido-4-methyl-9 pentonate also known as tetrahydrolipstatin. It is saturated derivative of the lipstatin a potent natural inhibitor of pancreatic lipase, isolated from the bacterium *Streptomyces toxytricini*. It acts by binding covalently with serine residue of the enzyme. On oral administration of orlistat with fat containing food, it hydrolyses triglyceride which reduces the absorption of monoacylglycerides and free fatty acids in to the body.¹

Determination of drug content in bulk and marketed formulation is essential step to confirm its purity and stability. To estimate drug content, simple and economic analytical method is required. In case of orlistat, few HPLC (High Performance Liquid Chromatography) methods are reported in literature. Stability indicating high performance liquid chromatographic method was developed to determine orlistat content in bulk and its degradation products.² Detection of the SSR impurity of orlistat in capsule by high performance liquid chromatographic method was reported.³ The new RP-HPLC method was reported to determine orlistat in bulk and pharmaceutical dosage form.⁴ For the quality control of Orlistat HPLC method was reported.⁵ There are some UV methods which are not reproducible. In case of HPLC method, it is costlier, not easily available and it requires skilled personnel to handle it. As compared to the HPLC method UV method is easy to perform and economical too. Stability-indicating stress degradation studies of Lafutidine using UV spectrophotometric method was carried out.⁶ Stress degradation study is performed as per ICH guideline at different stress conditions.⁷ From the literature review, it has been revealed that there is no stress degradation

study has been carried out by UV spectrophotometry method. However, there are certain limitations of the UV method such as, it is not possible to identify the impurity. Hence, major objective of the study was to develop UV method and to study stress degradation behavior of orlistat in different stress conditions.

MATERIALS AND METHODS

Instruments and materials

UV spectrophotometer (Inkarp – Sican 2301) was used. Orlistat was kind gift by the Biocon limited, Bangalore India. All chemicals and reagents used were of analytical grade.

Preparation of standard stock solution

Standard stock solution of 400µg/ml concentration was prepared by using methanol as solvent. It was filtered through whatmann filter paper #41 and used to prepare further dilutions.

Preparation of sample solutions

Aliquots of stock solution were subjected to serial dilutions with phosphate buffer pH-8 to get solutions with concentration of 8-28µg/ml.

Determination of λ_{max}

The wavelength of maximum absorbance (λ_{max}) of the drug was determined by scanning the drug solution with concentration 20µg/ml in range of 800nm-199nm. The drug solution was showing absorption maxima at

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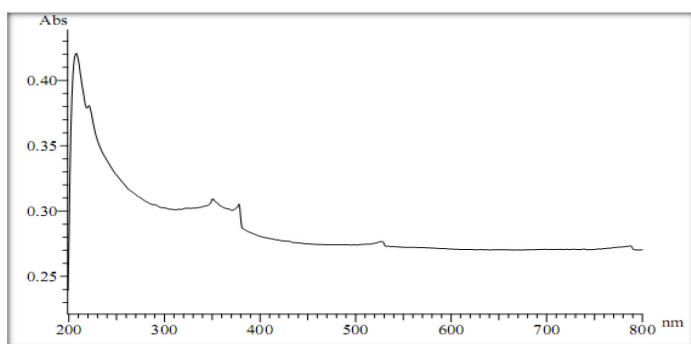


Figure 1 : UV spectrum of drug solution in Phosphate buffer pH-8.

205nm, so this wavelength was considered for further study. The UV spectrum of the drug solution is as shown (Figure 1).

Validation of method

Linearity

The linearity of measurements was evaluated by analyzing orlistat at different concentration. The calibration curve was obtained by plotting the absorbance versus the concentration data and was treated by linear regression analysis.

Precision

The precision of the method was studied in terms of intra-day changes in absorbance of drug solution on the same day and inter-day changes on three 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 formula for the LOD and LOQ are as follows

$$\text{LOD} = \frac{3.3\sigma}{s} \quad (1)$$

$$\text{LOQ} = \frac{10\sigma}{s} \quad (2)$$

Where σ = Standard deviation of the response,
s = Slope of the calibration curve

Accuracy (recovery method)

Accuracy of method was studied by recovery method. Recovery experiment was carried out by adding known amount of pure drug in powder mixture from capsule. Orlistat was added in level of 80%, 100% and 120%. Three samples for each level were analyzed and % recovery was calculated.

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 Lifesciences Pvt.Ltd.). Ten capsules were weighed and contents from capsule were obtained by removing capsule shell. Further, contents were mixed uniformly by using mortar and pestle. Quantity equivalent to 10mg of orlistat was weighed and transferred it into the 25ml of volumetric flask containing 15ml of methanol it was kept for 15 min with frequent shaking. Further volume was made up with the methanol and it was subjected for sonication on bath sonicator for 10 min. The solution was filtered through Whatman Filter Paper #41. This filtrate was diluted to get solution of the 20 μ g/ ml concentration.

The absorbance was measured against blank solution. Orlistat content of capsule was calculated using standard calibration curve. In addition, drug content was estimated in O-stat 60 (Aristo Pharmaceuticals Pvt. Ltd.).

Forced degradation study

Acid and alkali degradation

Acid degradation was performed into the 0.1N, 0.2N, 0.5N HCl and alkali degradation was carried out in 0.1N and 0.2N NaOH. The concentration of 20 μ g/ml orlistat was used for the degradation studies. Absorbance was measured at initial, 1st, 2nd, 3rd and 4th hr.

Neutral hydrolysis

To study degradation of orlistat in neutral conditions distilled water was used as solvent. Initially orlistat was dissolved in methanol and dilutions were made with distilled water.

Hydrogen peroxide degradation

Oxidative degradation was carried out by using 3% v/v and 30 % v/v hydrogen peroxide solutions as solvents. The samples were withdrawn at predetermined time intervals and analyzed after suitable dilutions at 205nm.

Photolytic degradation

Photolytic degradation was carried out by directly exposing orlistat to the sunlight during day time (60,000-70,000 lux) for 2 days.

RESULTS

Validation of method

Linearity was found to be 8-28 μ g/ml. Absorbance showed at different concentration was found to be linear with correlation coefficient 0.999 (Table 1). The Intra-day and Inter-day precision studies were carried out. The relative standard deviation was found to be 0.0029 and 0.0095 for Intra-day and Inter-day precision studies respectively. Intra-day and Inter-day precision is as shown in (Table 2). The LOD and LOQ of the developed method were found to be 0.3444 μ g/ml and 1.0740 μ g/ml respectively. Accuracy was studied by recovery method. Percentage accuracy was found to be 96.19% \pm 0.99% (Table 3).

Stress degradation study

Acid degradation was performed into the 0.1N, 0.2N, 0.5N HCl and alkali degradation was carried out in 0.1N and 0.2N NaOH. Absorbance of the solution was measured at the 205nm. From the Figure 2 decrease in absorbance was observed at 1h which concludes degradation of the drug in acidic stress condition. The drug was found to be unstable in alkaline condition. It showed absorbance 2.959 and 2.824 in 0.1N and 0.2N NaOH respectively. The results revealed that, degradation products are having more absorbance than drug. The neutral degradation was performed. More than 50% decrease in the absorbance was observed (Figure 2). It was found that orlistat is degraded within 1 h on neutral hydrolysis.⁵ Oxidative degradation was carried out by using 3%v/v and 30%v/v of hydrogen peroxide. Absorbance was increased up to the 10 which may due to the large amount of impurities produced after oxidation. Photolytic degradation study was carried out. Slight decrease in absorbance was observed at 1h which remained constant after 4 h. No extensive change in absorbance was measured.

Analysis of marketed capsule formulation

Analysis of two marketed formulations (O-STAT60 and OrLean- 60) was carried out by using developed method. Table 4 indicates the amount of orlistat in capsule samples was meeting requirement (95-105%) of label claim.

Table 1: Validation parameters.

Parameters	UV method observations
λ_{\max} (nm)	205
Linearity range ($\mu\text{g/ml}$)	8-28
Regression equation	$y = 0.027x - 0.113$
Correlation coefficient (r^2)	0.999
LOD ($\mu\text{g/ml}$)	0.3444
LOQ ($\mu\text{g/ml}$)	1.0740

Table 2: Results of precision study.

Sr No	Concentration ($\mu\text{g/ml}$)	Intra-day	Inter-day
1	20	0.400	0.385
2	20	0.401	0.391
3	20	0.394	0.401
4	20	0.402	0.395
5	20	0.401	0.405
6	20	0.400	0.38
Average	-	0.400	0.39
Standard Deviation	-	0.0029	0.0095

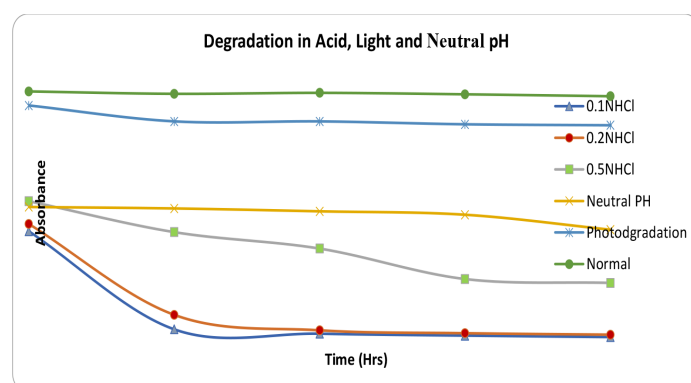
Table 3: Accuracy study by recovery method.

Name	Capsule amount*	Level of addition	Amount added	Total amount taken	Amount recovered	% recovery	Average % recovery
Orlistat	10	80	8	18	17.11	95.07	96.19
	10	100	10	20	19.39	96.98	
	10	120	12	22	21.23	96.53	

*-Amount equivalent to pure orlistat

Table 4: Orlistat content in marketed formulations.

Brand	Label claim	Amount found (Mean \pm SD, mg)	% Drug
O-STAT60 [®]	60	58.89 \pm 0.3	98.15
OrLean- 60 [®]	60	57.79 \pm 0.4	96.31

**Figure 2:** Degradation of orlistat in Acid, Light and Neutral pH.

DISCUSSION

The standard stock solution was prepared with concentration 400 $\mu\text{g/ml}$ by using methanol as solvent where, orlistat was found to be soluble. The solution was found to be clear, after filtration through Whatman filter paper #41, which was used for further dilutions.

The sample solutions concentration range was decided by scanning single dilution of concentration 20 $\mu\text{g/ml}$. The serial dilutions were carried out by using phosphate buffer pH-8 to get solutions with concentration of 8-28 $\mu\text{g/ml}$. The wavelength of maximum absorbance (λ_{\max}) of the drug was found to be 205nm when the solution was scanned at concentration 20 $\mu\text{g/ml}$. here the highest absorbance is observed at 205nm. Hence, 205nm wavelength was considered for further study. Souri *et al.* and Nama *et al.* detected orlistat at 205nm wavelength in their study. So, results obtained in this study are found to be in correlation with reported study.^{4,5} The absorbance of individual concentration was determined at 205nm wavelength. Here, absorbance was found to be increased in linear manner hence, method was found to be linear. The correlation coefficient supported linearity of the method as it is near to unity. To check the correctness of the method, the drug solution with specific concentration (20 $\mu\text{g/ml}$) was prepared and its absorbance was recorded at different time interval in a day and on different days. The intra-day and inter-day precision indicated correctness of method.

The Limit of Detection (LOD) and Limit of Quantification (LOQ) help to determine the sensitivity of the method, which is determined with deviation. From the LOD and LOQ study, it is observed that, the developed method can determine presence of drug and quantify it at very low concentration. The recovery analysis revealed that developed method was able to detect externally added known concentration of drug. It is evidenced that the method is accurate.

Accuracy of method was studied by recovery method

To evaluate the applicability of the developed analytical method at commercial scale it is applied and tested for its performance by testing some marketed formulations. The content in the commercially available marketed formulation of orlistat was determined by using developed UV spectrophotometric method. The marketed formulations were analyzed such as OrLean 60 from Eris Lifesciences Pvt.Ltd. and O-stat 60 from Aristo Pharmaceuticals Pvt. Ltd. The developed method, which was used to analyze the marketed samples, results obtained was found to be complying the specifications provided on label. Hence, the developed method was found to be useful in analyzing the commercial product.

The stability can be determined by exposing pure drug or (active pharmaceutical ingredient) at different stress conditions.⁶ From the stress degradation study, it is observed that the decrease in absorbance at 1h in acidic media, which concludes degradation of the drug in acidic stress condition. The stress degradation study, carried out in acidic media at 0.1N HCl and 0.2 N HCl, the sudden fall in absorbance was observed whereas in 0.5N HCl increase in absorbance was observed which is due to the increase in degradation products at highly acidic conditions. The drug was found to be unstable in alkaline condition as the absorbance of the drug supported the evidence. The results revealed that, degradation products are having more absorbance than drug. There is slight decrease in absorbance was observed at 1h, which remained constant after 4 h. Acid degradation study revealed that degradation of the drug in acidic condition whereas drug was found to be unstable in alkaline condition. The degradation at neutral pH was showed more than 50% decrease in the absorbance. Absorbance was increased up to the 10, which may due to the large amount of impurities produced upon oxidation. The photolytic degradation study revealed that slight decrease in absorbance at 1h, which remained constant after 4 h.

CONCLUSION

Stress degradation study was carried out for orlistat at different stress conditions like acid alkali, neutral, photolytic. Developed UV spectrophotometric method was found to be simple and economic. From stress degradation study it was found that, the orlistat was degraded in acid, alkali, neutral stress condition whereas it was not degraded in light. Hence, it is concluded that developed method is applicable for routine analysis in pharmaceutical industry.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

HCl: Hydrochloric acid; **NaOH:** Sodium Hydroxide.

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