Research Article

# Development and Validation of Derivative Spectroscopic Method for Determination of Orlistat in Bulk and Pharmaceutical Dosage Forms

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**ABSTRACT**

Derivative spectrophotometry involves the conversion of normal spectrum into its first, second and higher derivative spectra. Derivative spectrophotometric estimation was used for the elimination of irrelevant absorption. The drug Orlistat was determined at 202, 198, 196nm for zero, first and second order derivatives respectively using methanol as solvent. Linearity was obtained within the range of 20-70 µg/ml with correlation coefficient of 0.997, 0.999 and 0.999 for three order derivatives. The % recovery for the proposed method was found to be 101.24-128.3, 99.71-131.1 and 99.28-127.85% indicating no interferences from the capsule excipients. The result of analysis was validated statistically and recovery studies confirmed that the method was simple, efficient and reproducible derivative spectrophotometric method.

**Keywords:** Orlistat, Derivative spectroscopy, Zero order, First order, Second order, Orlistat by UV.

## INTRODUCTION

Orlistat, tetrhydrolipstatin, drug designed for treating obesity, chemically known as (2S)-1-[(2S,3S)-3-hexyl-4-oxooxetan-2-yl]tridecan-2-yl(2S)-2-formamido-4-methylpentanoate. It is marketed as a prescription under the trade name Xenical by Roche in most countries, and is sold over-the-counter as Alli. ORL is the saturated derivative of lipstatin a potent natural inhibitor of pancreatic lipases isolated from the bacterium *Streptomyces toxytricini*. Its primary function is preventing the absorption of fats from the human diet, thereby reducing caloric intake. It is intended for use in conjunction with a physician-supervised reduced-calorie diet[1]. Literature review reveals that determination of ORL by UV[2], RP-HPLC[3] and impurity determination by RP-HPLC[4]Literature review reveals that no derivative Spectra of Orlistat was developed till now. Derivative spectroscopy involves the measurement of Api without any matrix interferances[5]. Here by reporting a simple, rapid and reliable Derivative spectroscopic method for the estimation of ORL in bulk and pharmaceutical dosage forms. The structure of Orlistat was given in Figure 1.

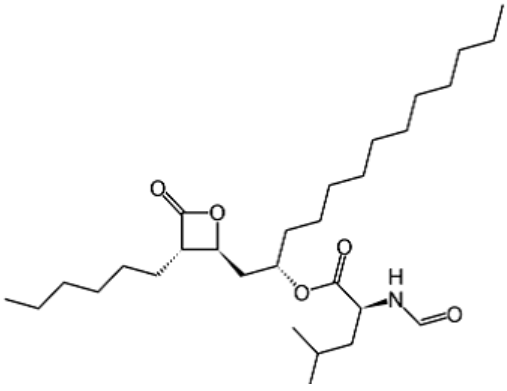

**Figure 1:** Structure of Orlistat

## MATERIALS AND METHODS

### Instrumentation

Spectral and absorbance measurements were done on UV Spectrophotometer with software UV Win, lab India. 10mm path length quartz cells were used. Digital analytical balance was used for weighing.

### Chemicals and Materials

Pure drug of Orlistat was obtained as a gift sample along with the certificate of analysis from RA CHEMICALS PVT LTD, Methanol (AR Grade) and Orlistat capsules were purchased from local market.

### Preparation standard stock solution

Weigh accurately 25mg of Orlistat was dissolved in few ml of methanol and the solution was diluted to 50ml. Further a 5ml solution was taken and again diluted to 25ml to obtain a standard stock solution of 100µgm/ml.

### Preparation test solution

A quantity of powder equivalent to 25mg of Orlistat (0.051mg) was accurately weighed and dissolved in 50ml of methanol, sonicate for 15 minutes and filtered through 0.45µ filter. The filtered solution was further diluted to obtain a concentration of 100µgm/ml.

### Validation

#### *Linearity*

Aliquots of solutions 2-7ml were taken from the standard stock solution in to 10ml volumetric flasks. The volume was made up to 10 ml using methanol to obtain the concentrations of 20, 30, 40, 50, 60 and 70 (mcg/ml). The absorbance was measured at 202nm, 198nm, 196nm against a blank as methanol. The linearity curve for zero order, first order and second order derivatives were

plotted was given in Figure 2-4. The spectrum for standard Orlistat solution in Figure 5-7.

***Precision (as per USP)***

Preparation of Precision sample solution (Prepare in six replicates):

Transfer carefully different concentrations of capsule content into 6 different 50mL volumetric flask and dissolve in 5mL methanol and make up the volume with methanol. From the above solution transfer 5ml to 25mL volumetric flask and make up to 25mL. Record the absorbance's at 202, 198, 196 nm against blank. The results were given in table 1 and in figures 2-4.

***Accuracy (as per USP)***

Accuracy can be done by measuring the absorbance of three replicate samples at 100% dilution and three replicate samples each at other levels prepared by spiking Orlistat API Sample solution at 10%, 20%, 30%, to test stock solution of target concentration level.

Preparation of sample solution (100% Dilutions) Prepare in triplicate:

A quantity of powder equivalent to 51.6mg of Orlistat transfer carefully into 50mL volumetric flask and dissolve in 5mL methanol and make up the volume with methanol. From the above solution transfer 5ml to 25mL volumetric flask and make up to 25mL.

Preparation of sample solution (10% standard spiked) Prepare in triplicate:

From the above sample stock solution transfer carefully 5ml into 25ml volumetric flask, to this add 0.5ml of standard stock solution and make up the volume with methanol.

Preparation of sample solution (20% standard spiked) Prepare in triplicate:

From the above sample stock solution transfer carefully 5ml into 25ml volumetric flask, to this add 1.0ml of standard stock solution and make up the volume with methanol.

Preparation of sample solution (30% standard spiked) Prepare in triplicate:

From the above sample stock solution transfer carefully 5ml into 25ml volumetric flask, to this add 1.5ml of standard stock solution and make up the volume with methanol.

**Table 1:** Precision Data of orlistat

| S.No. | Standard Absorbance | | | Sample Absorbance | | | Mg/ Capsule | % of Drug |
|---|---|---|---|---|---|---|---|---|
| | "0" order | 1st order | 2nd order | "0" Order | 1st order | 2nd order | | |
| 1 | 0.943 | 0.121 | 0.034 | 0.946 | 0.124 | 0.034 | 60.12 | 100.24 |
| 2 | 0.943 | 0.121 | 0.034 | 0.935 | 0.115 | 0.030 | 59.93 | 99.86 |
| 3 | 0.943 | 0.121 | 0.034 | 0.951 | 0.129 | 0.035 | 60.24 | 100.48 |
| 4 | 0.943 | 0.121 | 0.034 | 0.943 | 0.121 | 0.034 | 60.00 | 100.00 |
| 5 | 0.943 | 0.121 | 0.034 | 0.930 | 0.110 | 0.028 | 59.87 | 99.74 |
| 6 | 0.943 | 0.121 | 0.034 | 0.939 | 0.118 | 0.032 | 59.96 | 99.20 |
| **Mean** | **0.943** | **0.121** | **0.034** | **0.9406** | **0.1195** | **0.0321** | ------ | **99.92** |
| **SD** | ----- | ----- | ----- | **0.0076** | **0.0067** | **0.0027** | ------ | ------ |
| **%RSD** | ----- | ----- | | **0.807** | ----- | | ----- | ----- |

**Table 2:** Accuracy Data of orlistat

| S. No | No of Replicates | | | Absorbance | | | Average | | | % Recovery | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | "0" order | 1st order | 2nd order | "0" order | 1st order | 2nd order | "0" order | "1st" order | 2nd order | "0" order | 1st order | 2nd order |
| 1 | | 100%-1 | | 1.025 | 0.131 | 0.038 | 1.026 | 0.131 | 0.038 | 101.24 | 99.71 | 99.28 |
| | | 100%-2 | | 1,028 | 0.131 | 0.039 | | | | | | |
| | | 100%-3 | | 1.027 | 0.130 | 0.038 | | | | | | |
| 2 | | 110%-1 | | 1.049 | 1.135 | 0.039 | 1.049 | 0.135 | 0.039 | 109.88 | 111.1 | 106.42 |
| | | 110%-2 | | 1.050 | 1.135 | 0.040 | | | | | | |
| | | 110%-3 | | 1.048 | 1.135 | 0.039 | | | | | | |
| 3 | | 120%-1 | | 1.076 | 0.138 | 0.040 | 1.075 | 0.138 | 0.041 | 119.66 | 119.71 | 120.71 |
| | | 120%-2 | | 1.075 | 0.138 | 0.041 | | | | | | |
| | | 120%-3 | | 1.074 | 0.138 | 0.041 | | | | | | |
| 4 | | 130%-1 | | 1.099 | 0.142 | 0.042 | 1.098 | 0.142 | 0.042 | 128.3 | 131.1 | 127.85 |
| | | 130%-2 | | 1.098 | 0.141 | 0.042 | | | | | | |
| | | 130%-3 | | 1.098 | 0.142 | 0.042 | | | | | | |

**Table 3:** Assay Data of Orlistat.

| Sample Id | Zero order | Mg/cap | First order | Mg/cap | Second order | Mg/cap |
|---|---|---|---|---|---|---|
| 1 | 105% | 0.0639 | 103% | 0.062 | 0.0623 | 103% |

All the above solutions were filtered through 0.45µ filter and measure the absorbances at 202, 198, 196 nm. The results were given in table 2 and figures 5-7.

### Assay

The proposed method was applied to the determination of Orlistat in the brand name zero fat. The spectra for zero order, first order and second order derivatives were obtained. The results were given in table 3.

## RESULTS

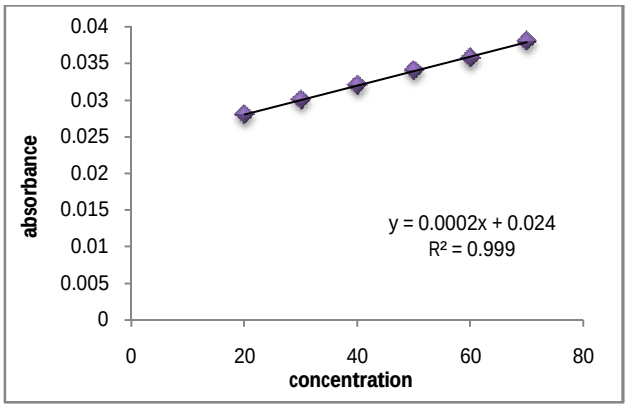


**Figure 2:** Zero Order linearity plot of Orlistat

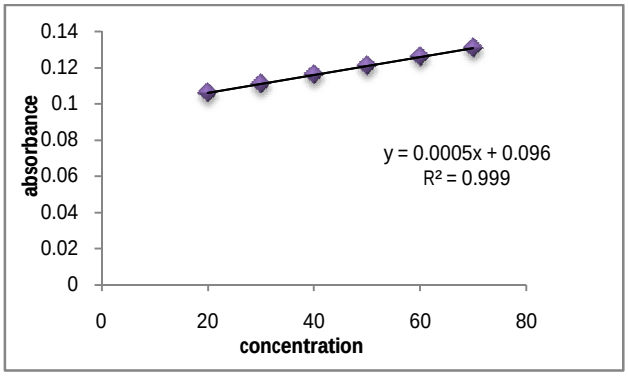


**Figure 3:** First Order linearity plot of Orlistat

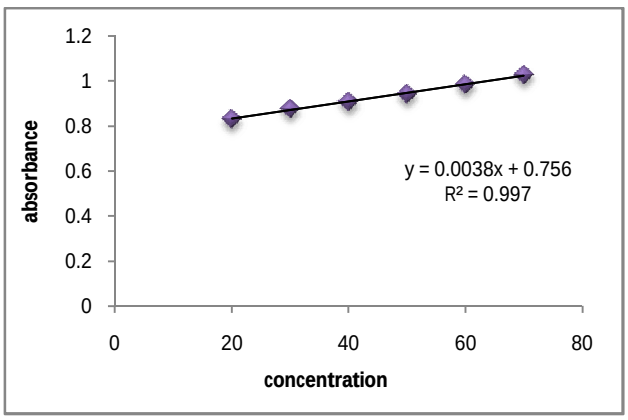


**Figure 4:** Second Order linearity plot of Orlistat

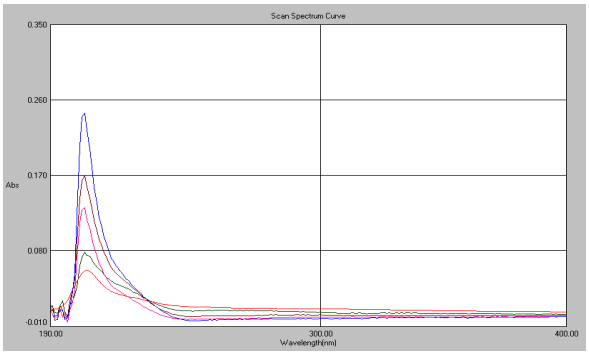


**Figure 5:** All Spectra of Orlistat (zero order)

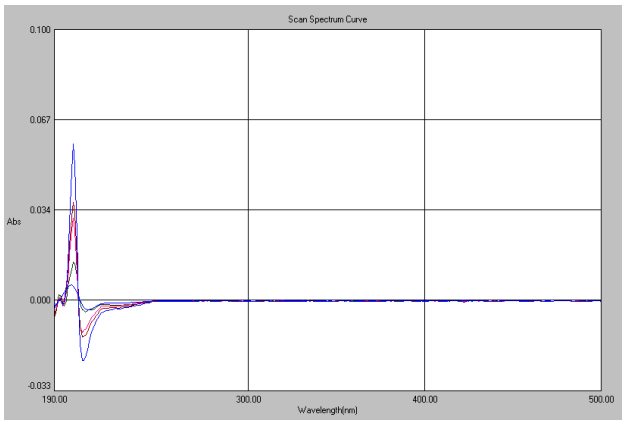


**Figure 6:** All spectra of Orlistat (First order)

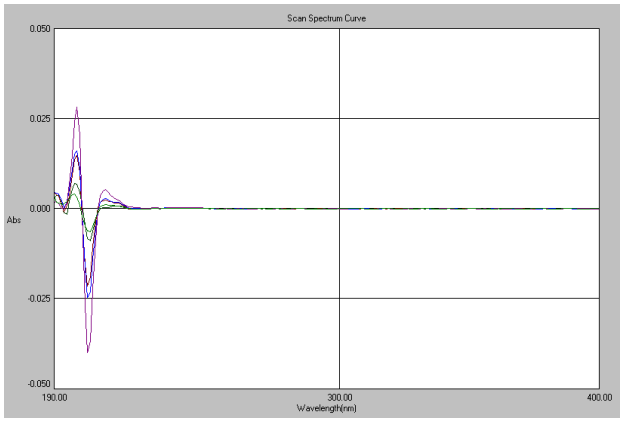


**Figure 7:** All spectra of Orlistat (Second order)

## CONCLUSION

The method was validated and found to be simple, sensitive, accurate and precise as per USP guidelines. Zero order, first order, second order derivative spectrophometric methods were developed for the determination of Orlistat in bulk and capsule dosage form. Orlistat can be directly determined in capsules in presence of excipients without sample pre treatment procedures by using spectrophotometric methods.

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