RESEARCH ARTICLE

Differential derivative method development and Validation of Orlistat by UV: A Spectrophotometric Technique

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ABSTRACT

The aim of the present research work was to develop accurate and rapid Differential derivative spectrophotometric method. The stock solution was prepared by taking methanol as solvent. Further dilutions were made by using equimolar solutions with 0.1N NaOH and 0.1N HCl separately and the difference in absorptivities was measured at wavelengths of 214 nm. It was proved that spectrum differentiation significantly corrects errors resulting from overlapping background. Difference in absorbance between maxima and minima was calculated to find out the amplitude. The amplitude was plotted against concentration. Beer's-Lambert's law is valid in the concentration range 10-30 for zero, first and second orders. The result of analysis was validated statistically and by recovery studies.

Keywords: Orlistat (ORL), 0.1N NaOH, 0.1N HCl, Differential Spectroscopy, derivatives, UV Spectroscopy

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.

In Differential derivative, the test and standards were prepared in different solvents based on their solubility where the solution which has lower λmax has taken as sample, which was measured against blank showing higher λ_{max} [2]. This method is adaptable only for pH insensitive substances. The spectrum obtained was transformed into their respective derivatives by optical techniques which helps in exact determination of API without matrix interferences. [3]

Literature review reveals that determination of ORL by RP-HPLC [4] and impurity determination by RP-HPLC [5] but there was no differential derivative Spectra of Orlistat was developed till now. 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 Figure1.

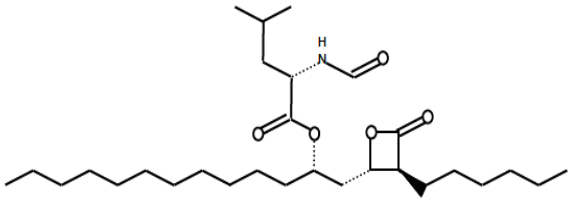

Figure 1: Structure of Orlistat

MATERIAL AND METHODS

Instrumentation:

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

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Chemicals and Materials:

Pure drug of Orlistat was obtained as a gift sample along with the certificate of analysis from RA CHEMICALS PVT LTD, Hyderabad. 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, sonicated for 15 minutes and filtered through 0.45µ filter. The filtered solution was further diluted to obtain a concentration of 100µgm/ml.

Selection of Analytical wavelength:

From stock solution of 100 µg/ml, take 1ml-1ml into two volumetric flasks and then make up the volume in one volumetric flask with 0.1N NaOH and in another with 0.1N HCL up to 10ml.Then spectra was scanned within range of 190-800nm by keeping acidic form (i.e. ORL in 0.1N HCL) in reference cell and basic form (i.e. ORL in 0.1N NaOH) in sample cell as blank.

Assay

The proposed method was applied to the determination of Orlistat in the brand name zero fat. The results were given in table 1.

Validation:

Linearity & Range

Calibration curves were plotted for each spectrophotometric method. The statistical parameters and regression equations were calculated from the calibration curves along with the standard error of the slope and the intercept as shown in Table 1. Regression analysis indicated a linear relationship between amplitude and concentration, within the ranges of 10 - 30 µg/ml for zero, first and second order derivatives (Table 2). All spectra were shown in figure 2, 3, 4.

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 dissolved 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.

Further dilutions were made to get the final concentration of 20µg/ml both in 0.1N NaOH and 0.1N HCl individually and Record the absorbance for zero, first, second order derivatives. The results were given in table 3.

Accuracy: as per USP:

Accuracy can be done by measuring the absorbance of 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. All the above solutions are filtered and measure the absorbance. The results were given in table 4.

Detection limit

The Detection Limit of an individual analytical procedure was the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The detection limit (LOD) may be expressed as:

$$LOD = 3.3\sigma / S$$

Where

σ = Relative standard deviation of the response.

S = the slope of the calibration curve (of the Analyte).

Quantification limit

The Quantification limit of an analytical procedure was the lowest amount of analyte in a sample, which can be quantitatively determined with suitable

precision and accuracy. The Quantification Limit (LOQ) may be expressed as:

$$LOQ = 10\sigma/S$$

Where

σ = Relative standard deviation of the response.

S = the slope of the calibration curve (of the analyte).

The results were tabulated in table 5.

Ruggedness and Robustness

Ruggedness was determined by performing the same proposed method on different instruments, by two different analysts and on different days to check the reproducibility.

Robustness was the capacity of a method to remain unaffected by small deliberate variations in method parameters. The results were tabulated in table 6, 7.

RESULTS AND DISCUSSION

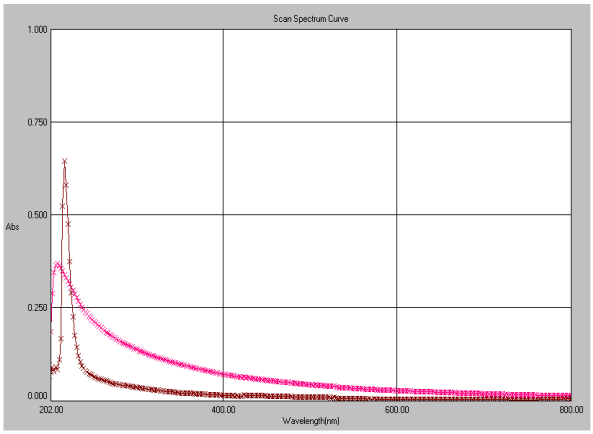

Fig.2: All Spectra of Orlistat (Zero order)

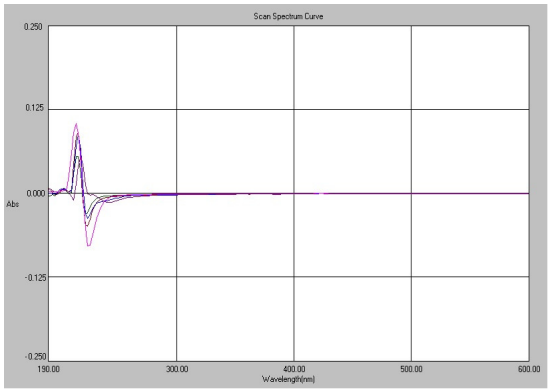

Fig.3: All Spectra of Orlistat (First order)

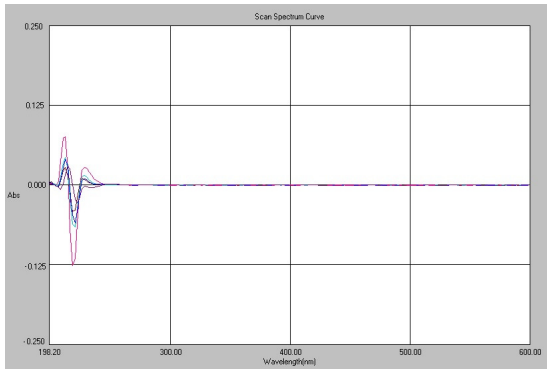

Fig.4: All Spectra of Orlistat (Second order)

Assay data of Orlistat:

Table 1: Assay of Capsule formulation

Method	Label claim	Standard Absorbance	Sample Absorbance	Mg/cap	Percentage	SD	%RSD
Zero order	60	0.649	0.857	59.07	98.46	0.322	0.327

Linearity data of Orlistat

Table 2: Linearity data of Orlistat

Methods	slope	Intercept	r^2
Method A			
0	0.017	0.118	0.9992
1st	0.0023	0.0408	0.9998
2nd	0.0021	0.0206	0.9997

Precision data of Orlistat

Table 3: Precision data of Orlistat

Method	Conc (µg/ml)	Intermediate %RSD		Avg	Inter day %RSD (Days)			Avg	Repeatability %RSD(h)			Avg
		Instr I	Instr II		1	2	3		0	8	16	
0	20	0.81	1.01	0.91	0.59	1.01	1.11	0.90	0.22	0.81	1.14	0.72
1st	20	1.74	1.46	1.60	1.69	1.82	1.50	1.67	1.42	1.48	1.38	1.43
2nd	20	1.86	1.68	1.77	1.82	1.44	1.55	1.60	1.68	1.88	1.64	1.73

Accuracy data of Orlistat

Table 4: %Recovery studies from capsule formulation

Method	Conc Level	Amount Added	Amount Recovered Mean	%Recovery Mean	SD	%RSD
0	110	0.5	0.55	110.63	0.663	0.599
	120	1	1.20	120.78	0.415	0.344
	130	1.5	1.96	131.16	0.415	0.316
1st	110	0.5	0.55	110.70	0.508	0.459
	120	1	1.19	119.65	0.508	0.425
	130	1.5	1.93	128.75	0.508	0.394
2nd	110	0.5	0.54	109.81	0.658	0.599
	120	1	1.19	119.69	0.569	0.476
	130	1.5	1.95	130.33	0.658	0.504

LOD & LOQ

Table 5: LOD & LOQ data of Orlistat

Method	LOD	LOQ
Zero Order	0.19665	0.59591
First Order	0.585748	1.771993
Second Order	0.641533	1.944039

RUGGEDNESS & ROBUSTNESS

Table 6: Ruggedness data

Methods	Ruggedness					
	Analyst I			Analyst II		
	Mean	SD	%RSD	Mean	SD	%RSD
0	0.461	0.34	0.34	0.462	1.00	1.01
1st	0.080	1.18	1.28	0.079	1.74	1.90
2nd	0.052	1.32	1.59	0.052	1.56	1.88

Table 7: Robustness data

Methods	Robustness					
	Room Temp			25ºC		
	Mean	SD	%RSD	Mean	SD	%RSD
0	0.462	0.462	0.462	0.462	0.462	0.462
1st	0.079	0.079	0.079	0.079	0.079	0.079
2nd	0.056	0.056	0.056	0.056	0.056	0.056

CONCLUSION

Difference spectrophotometric technique helpful for discriminating the determination of API in multiple solvents where its derivatives help in measuring the API without matrix interferences. Hence this method is simple, precise, accurate and economic.

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