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Original Research Paper

METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF ORLISTAT BULK DRUG USING HYDROXYLAMINE SOLUTION BY VISIBLE SPECTROSCOPY

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ABSTRACT

A simple, precise, accurate visible spectroscopic method was developed and validated for the determination of Orlistat by using Hydroxylamine reagent and ferric chloride solution in co-solvent ethanol and subsequently diluted with distilled water. At the λ_{max} of 515 nm, it was proved linear in the range 5-15 µg/ml, and exhibit good correlation coefficient (R^2 =0.9978) and excellent mean recovery (98.82-102.3% w/w). The developed method was validated for accuracy, precision, robustness, ruggedness according to ICH guidelines. All these parameters showed adaptability of the method for the quality control analysis of Orlistat API.

Keywords: Orlistat, Hydroxylamine, Ferric chloride, Visible spectrophotometry, Validation.

INTRODUCTION

Orlistat is (S)-2-formylamino-4-methyl-pentanoic acid (S)-1-[[(2S, 3S)-3-hexyl-4-oxo-2-oxetanyl] methyl] dodecyl ester.[1] It is a potent, specific, and long-acting inhibitor of gastrointestinal lipases. It exerts its therapeutic activity in the lumen of the stomach and small intestine by preventing the gastric and pancreatic lipases from hydrolyzing dietary fat, in the form of triglycerides, into absorbable. There was no reported method for estimation of Orlistat by Visible spectroscopy. This method is based on the formation of hydroxamic acid by the reaction of amide present in orlistat with hydroxyalamine in alkaline medium and a colour developing step involving the reaction of ferric ion with the hydroxamic acids in acidi medium.[2] The absorbances of the final dilutions were measured at 515 nm because of formed iron complexes.[3] Literature survey revealed that several methods like UV spectrophotometry[4,5,6] and HPLC[7,8] have been reported. The aim for undertaking the present work is to develop a visible spectroscopic method for the estimation of Orlistat based on the presence of amide functional group.

EXPERIMENTAL

Materials and Methods

Instrument

UV-Visible Spectrophotometer (PG instruments T60), UV-Visible Spectrophotometer (Lab India, 3000+), 'Fast Clean' Ultra sonicator.

Chemicals Required

Orlistat drug sample was supplied as gift sample by Murali Krishna Pharma Pvt. Ltd., Pune, Maharashtra and all other chemicals like Hydroxylamine, conc. Hydrochloric acid, ferric chloride, sodium chloride were used which are Purchased from E. Merck (India) Ltd.

Preparation of Reagents

3.5 N Sodium hydroxide solution: Weigh accurately about 7 gm of sodium hydroxide pellets and dissolve in 50 ml of distilled water.

2 N Hydroxylamine HCL reagent: Weigh accurately about 6 gm of hydroxylamine HCl and dissolve in distilled water and sonicate it for 20 min and makeup to volume 50 ml with distilled water. Then mix the sodium hydroxide solution and hydroxylamine HCL solution.

0.74 N Ferric chloride solution in 0.1 N HCL: Weigh accurately 1.2 gm of $FeCl_3$ and dissolve in 10 ml 0.1N HCL solution.

Procedure for Standard Solution and Standard Curve

Weigh accurately 50 mg of Orlistat and dissolve in 50 ml of ethanol (1000 µg/ml). From that pipette out 0.5 ml into a 10 ml volumetric flask and add 2 ml of Hydroxylamine reagent and keep for 3 hrs at 50^0C. After the reaction time add 1 ml $Fecl_3$ and 1 ml 3.5 N HCL solution and makeup the volume up to 10 ml with distilled water (50 µg/ml). From this solution prepare serial dilutions of 5, 7.5, 10, 12.5, 15 µg/ml by pipetting out 1 ml, 1.5 ml, 2 ml, 2.5 ml and 3 ml into 5 different 10 ml volumetric flasks respectively and makeup the volume to 10ml with distilled water. Then measure the absorbances of 5, 7.5, 10, 12.5, 15 µg/ml solutions at 515 nm.

Assay Procedure

Weigh accurately 50 mg of Orlistat and dissolve in 50 ml of ethanol (1000 µg/ml). From that pipette out 0.5 ml into a 10 ml volumetric flask and add 2 ml of Hydroxylamine reagent and keep for 3 hrs at 50^0C. After the reaction time add 1 ml $Fecl_3$ and 1 ml 3.5 N HCL solution and makeup the volume up to 10 ml with distilled water (50 µg/ml). From this solution pipette out 2 ml solution and makeup the volume to 10 ml with distilled water. Then measure the absorbances at 515 nm. Calculate the content of Orlistat from the data derived from calibration curve.

METHOD VALIDATION[9]

The method was validated for specificity, precision, linearity, accuracy, LOD, LOQ, ruggedness and robustness by the following procedures.

Linearity

The linearity of calibration curve (absorbance vs. concentration) for the drug solution was checked over the concentration ranges of about 5-15 µg/ml for Orlistat. The correlation coefficient and equation of the regression analysis were obtained. The linearity data was plotted.

Precision

Three concentrations of 10 µg/ml were prepared by transferring 2 ml from the 50 µg/ml solution into 10 ml volumetric flask and make up the volumes to the mark with distilled water and the absorbances were read at 515 nm. The % RSD was calculated and the %RSD < 2%.

% RSD = Amount found /Amount added*100

Accuracy

Recovery studies were carried out by pure drug solution at three different concentration levels (50, 100 and 150 %). From the 50 µg/ml solution pipette out 1 ml, 2 ml, 3 ml into three different 10ml volumetric flasks and make up the volume up to the mark with distilled water in order to get 50, 100, 150% concentration levels. These solutions were analyzed by using distilled water as blank. The amount of drug was estimated by measuring absorbance at different spiked levels. The recovery was verified by estimation of drug in triplicate.

% Recovery = Standard deviation / mean of measurements*100

Limit of Detection and Limit of Quantification

The LOD and LOQ of Orlistat were determined by using standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines. LOD and LOQ values were calculated using the relation,

$LOD=3.3\delta /S$

$LOQ=10\ \delta /S$

Robustness

The evaluation of robustness should be considered during the development phase and to check the assay procedure is unaffected by small change in procedure. The sample solution of 10 µg/ml was prepared in triplicates and the absorbances were measured in different variable conditions like change in wavelength, change in

volume makeup etc. The % RSD values of the results corresponding to the absorbance were expressed.

Ruggedness

The ruggedness of an analytical method was the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of test conditions like different analysts, different laboratory, different instrument, different lots of chemical etc. The sample solution of 10 µg/ml was prepared in triplicate and the absorbances were measured. The % RSD of the results analogous to the absorbances were expressed.

RESULTS

Table1: Results for calibration curve

Concentration	Absorbance
5 µg/ml	0.0045
7.5 µg/ml	0.062
10 µg/ml	0.084
12.5 µg/ml	0.099
15 µg/ml	0.118

Table 2: Parameters for calibration curve

Parameter	Observations
Calibration curve	Linear
Expression	Y=mX+c
Factor (m)	0.0073
Factor(c)	0.0084
Correlation coefficient(r^2)	0.9975

Limit of Detection and Limit of Quantification

LOD=3.3 σ/slope

Where, σ = standard deviation of blank responses

S = slope of calibration curve for linearity

Table 3: Blank responses

Concentration	Responses
0 µg/ml	0.049
0 µg/ml	0.049
0 µg/ml	0.048
Standard deviation	0.000577

σ = 0.000577

S = 0.0073

LOD = 3.3 * 0.000577 / 0.0073 = 0.261 µg/ml

LOQ = 10 σ/slope

Where, σ = standard deviation of blank responses

S = slope of calibration curve for linearity

Table 4: Blank responses

Concentration	Responses
0 µg/ml	0.049
0 µg/ml	0.049
0 µg/ml	0.048
Standard deviation	0.000577

σ = 0.000577

S = 0.0073

LOQ = 10 * 0.000577 / 0.0073 = 0.791 µg/ml

Precision

Table 5: Results for precision

Concentration	Absorbance
10 µg/ml	0.084
10 µg/ml	0.084
10 µg/ml	0.083
Mean	0.084
Standard Deviation	0.000577
%RSD	0.686

Accuracy

Table 7: Recovery studies for Orlistat

Concentration	Absorbance			Mean	Amount found	% Recovery
50%(5 µg/ml)	0.046	0.045	0.045	0.045	5.01	100.2
100%(10 µg/ml	0.082	0.083	0.082	0.082	10.08	100.8
150%(15 µg/ml)	0.120	0.120	0.119	0.120	15.2	101.3

Robustness

Table 8: Change in wavelength

Concentration	Wavelength	Absorbance
10 µg/ml	514nm	0.095
10 µg/ml	515nm	0.094
10 µg/ml	516nm	0.094
Mean		0.094
Standard Deviation		0.000577
%RSD		0.163

Table 9: Change in volume added

Volume added	Absorbance			Mean
1.9ml	0.088	0.088	0.088	0.088
2ml	0.088	0.090	0.089	0.089
2.1ml	0.090	0.091	0.091	0.091
Mean				0.089
Standard deviation				0.001528
% RSD				1.71

Table 10: Ruggedness

	Analyst-1	Analyst-2
Concentration	Absorbance	Absorbance
10 µg/ml	0.089	0.086
10 µg/ml	0.088	0.085
10 µg/ml	0.088	0.086
% RSD	1.7807	

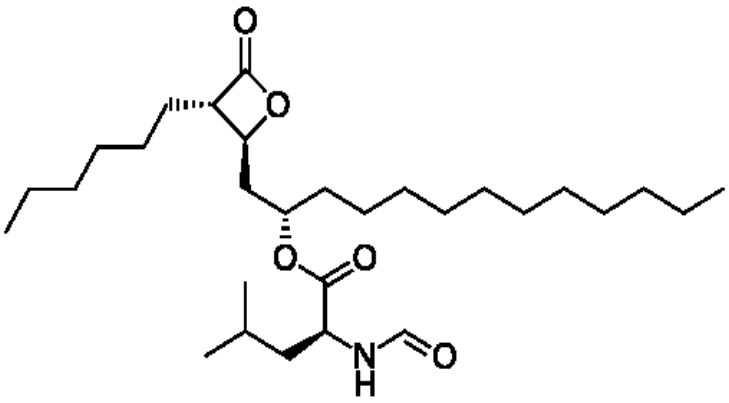

Figure 1: Structure of Orlistat

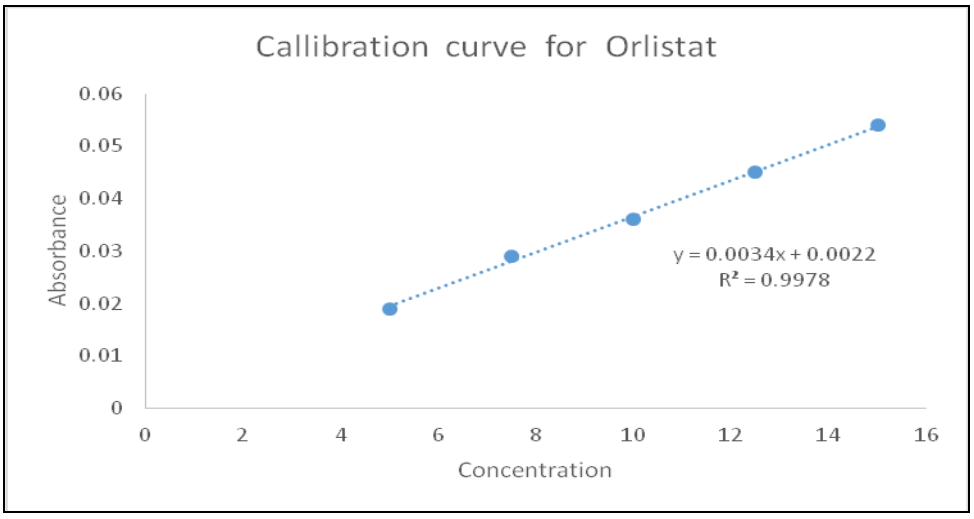

Figure 2: Callibration curve for Orlistat

DISCUSSION

A simple, precise and accurate visible spectrophotometric method has been developed and validated for estimation of Orlistat. The proposed method was validated as per ICH Q2 (R1) guidelines for precision, linearity, accuracy and recovery.

Linearity

The absorbance were observed from 5 to 15 µg/ml were shown in Table 1. Linearity was obtained between 5 to 15 µg/ml. Calibration graph was plotted for concentration and absorbance. The equation of calibration curve Figure 2 obtained was y = 0.0073x + 0.0084. The correlation coefficient (r) was 0.9975 shown in Table 2.

Precision

Three determinations of 10 µg/ml was prepared and the corresponding absorbances were observed Table 5. The % RSD was found to be 0.686.

Accuracy

Accuracy of the method recovery was performed by standard addition method. The recovery was performed at three levels 50%, 100% and 150% of standard Orlistat. Solutions were analyzed and percentage recovery was found between100.2 to 101.3 %w/v and was shown in Table 7.

Limit of Detection and Limit of Quantification

Limit of Detection and Limit of Quantification were found to be LOD = 0.261 µg/ml, LOD = 0.791 µg/ml respectively.

Robustness

Triplicate of 10 µg/ml was prepared at different experimental conditions like change in sample concentration and change in wavelength and their corresponding absorbances were noted. The % RSD was found to be 1.71, 0.613 respectively for the variable parameter like change in added volume, change in wavelength.

Ruggedness

Triplicate of 10 µg/ml was prepared by different analysts and their corresponding absorbance were noted. The % RSD was found to be 1.7807 for different Analyst.

Conclusion: A validated Visible spectroscopic method has been developed for the determination of Orlistat in bulk drug. The developed method is precise, accurate, robust and specific.

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REFERENCES

1. Heckelman, A; Neil, O and Smith, M J (2001), "***The Merck Index : An Encyclopaedia of Chemicals, Drugs and Biologicals***", White House Station, New Jersey, 1228.
2. Einar, Brochmann; Hanffen, hanssen and Takery Higuchi, '***Pharmaceutical Analysis'***, CBS Publications & Distributors, New Delhi, 547-550.
3. FE Critchfield, "***Organic Functional Group Analysis***", Vol-8, 53-54.
4. Hemant Kumar, T; Srinivasa Rao, Y; Teja Lakshmi, G and Vara Prasada Rao, K (2015), "Visible Spectrophotometric Methods for the Estimation of Orlistat in Bulk and Pharmaceutical Dosage Form", ***J. Pharm. Sci. & Res.***, Vol. 7 (3), 155-158.
5. Dhanalakshmi, K; Nagarjuna Reddy, G; Priyanka, gaddam and Sreenivasa, S (2013), "Estimation of Orlistat by various Spectrophotometric Techniques", ***Indo American Journal of Pharmaceutical Research***, Vol 3, Issue 7, 5212-5220.
6. Hemant Kumar, T; Manasa Reddy, K; Prasanna Kumar, R and Rishika, D (2011), "***International Journal of Science and Research***" Vol. 2 (9), 2469-2471.
7. Ghias, L; Haririan, I; Mohammadia, A; Rezanour, N and Walker, RB (2006), "A stability indicating high performance liquid chromatographic assay for the determination of Orlistat in capsules", ***Journal of Chromatography A***, 1116 153–157.
8. Basavasewara Rao, MV; Muralidhar, P; Reddy, BCK and Srinivas Rao, T (2008), "Reverse Phase High Performance Liquid Chromatographic determination of Orlistat Pellets 50.0%", ***Rasayan J. Chem***, Vol.1, No.3, 636-638.
9. "***ICH Harmonized Tripartite Guidelines***", Validation of analytical procedures: text & methodology, Q2 (R), 2005.

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