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Visible Spectrophotometric Methods for the Estimation of Orlistat in Bulk and Pharmaceutical Dosage Form

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Abstract
Two simple and reproducible visible spectrophotometric methods were developed and validated for determination of Orlistat in bulk and capsule dosage form. Method A is the extractive spectrophotometric method based on formation of ion-pair complexes of Orlistat with Solochrome Black-T forming a pink colored chromogen in which the absorption maxima found at 546 nm. The linearity range was determined in the concentration range of 5-30 µg/mL with correlation coefficient (r^2) 0.999. Method B, involves a charge transfer complex with 0.5 % w/v iodine solution in the linearity range of 2-10 µg/mL with correlation coeeficient (r^2) 0.999 and the spectra was determined at 443 nm. The developed method was validated as per ICH guidelines.Recovery studies gave satisfactory results indicating that none of the major additives/excipients interferred with the assay method. This method may be useful for routine laboratory analysis of Orlistat.

Keywords:
Orlistat, Solochrome Black-T, 0.5% w/v Iodine solution, Visible Spectrophotometric, Validation.

INTRODUCTION

Orlistat[1] is (S)-2-formylamino-4-methyl-pentanoic acid (S)-1-[[(2S, 3S)-3-hexyl-4-oxo-2-oxetanyl] methyl]dodecyl ester (**Fig.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[2-6] from hydrolysing dietary fat, in the form of triglycerides, into absorbable free fatty acids and monoglycerides. There is dearth of analytical methods reported in Literature [7-10] but no methods have been reported in visible spectrophotometric method for estimation of orlistat in bulk and pharmaceutical dosage form. The reported analytical techniques require expensive experimental set-up and are not affordable in every laboratory for routine analysis. Although visible spectrophotometric methods are the instrumental methods of choice commonly used in industrial laboratories, for their simplicity, selectivity and sensitivity, only few reports are there for the determination of orlistat in dosage forms. Therefore, the need for a fast, low cost and selective method is obvious, especially for routine quality control analysis of pharmaceutical products containing orlistat. The main objective of the present study was to develop simple, accurate, precise, and economic visible spectrophotometric methods for estimation of Orlistat in bulk and pharmaceutical formulation.

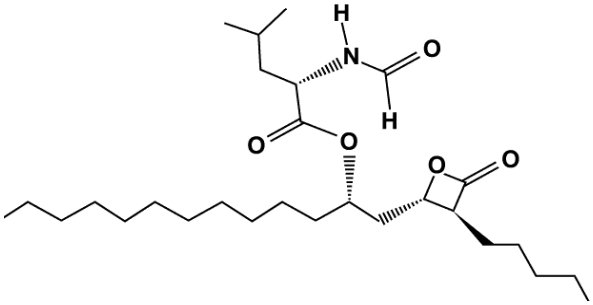

Fig.1 Chemical Structure of Orlistat

MATERIALS AND METHODS

Instrumentation

All spectral measurements were made on ELICO UV/VIS SL-210 Double beam UV-Visible spectrophotometer with 1cm matched quartz cells. The spectra were recorded by using spectral treats software. A Elico LI 210 p^H meter was used for p^H measurements.

Reagents and Chemicals

All reagents and chemicals used were of analytical grade purity. Isopropyl alcohol, double distilled water and methanol was obtained from Merck India Ltd. Dichloromethane AR grade was used. The details for the three methods are as follows

0.1 % w/v Solochrome Black T solution (Finar) was prepared by dissolving 100 mg of Solochrome Black-T in 100 mL of double distilled water.

KCl-HCl Buffer (p^H 2.0) was prepared by addition of 50 mL of 0.2 M KCl and 13 mL of 0.2 M HCl in 100 mL volumetric flask and then double distilled water was added up to the volume.

0.2 M HCl (Merck) was prepared by diluting 17.3 mL con.HCl to 1000 mL with double distilled water.

0.2 M KCl (Merck) was prepared by dissolving 14.911 gms of KCl in 1000 mL of double distilled water.

0.5% w/v Iodine solution (Merck) was prepared by dissolving 1.25 g of iodine in 250 mL of dichloromethane.

Drug Sample

The pharmaceutical grade Orlistat sample was gifted from R A Chem Ltd. Hyderabad. Capsule formulation Zerofat™-60 (Orlistat capsules) contains Orlistat 60 mg was used in present study.

Preparation of working standard drug solution

Method A and Method B

The Standard 100 mg of Orlistat was weighed accurately and transferred to 100 mL volumetric flask. It was

dissolved properly and diluted up to the mark with methanol to obtain concentration of 1000 µg/mL. From this 10 mL was transferred into 100 mL volumetric flask and diluted with methanol to get concentration of 100 µg/mL.

Procedure for Calibration curve

Method A

Aliquots of (0.5-3 mL) standard stock solution (100 µg/mL) of Orlistat were transferred into a series of 10 mL calibrated volumetric flask. To each of the aliquots, 2.0 mL of KCl-HCl buffer(p^H 2.0) was added followed by addition of 2.0 mL of (0.1% w/v) solochrome black-T and kept aside for 2 min with occasional shaking for the completion of reaction at the room temperature. The volumes were made up to 10 mL with methanol. And then the above solutions were extracted with 10 mL of chloroform in separating funnel for 5 min where the lower organic layer was collected in which anhydrous sodium sulphate was added and shaken for few seconds. Finally, the absorbance of the solution was measured at 546 nm against the reagent blank. The absorption spectra and calibration curve was shown in **Fig.2** and **Fig 4** respectively.

Method B

Aliquots of (0.2-1 mL) standard stock solution (100 µg/mL) Orlistat were transferred into a series of 10 mL calibrated volumetric flask. To each of the aliquots 1.2 mL of (0.5% w/v) iodine solution was added and kept aside for 20 min at room temperature. The volumes were made up to 10 mL with dichloromethane and the absorbance of each solution was measured at 443 nm against the reagent blank. The absorption spectra and calibration curve was shown in **Fig.3** and **Fig.5** respectively.

Estimation of Orlistat in capsule formulation

Accurately 20 capsules of Orlistat were weighed. Capsule powder equivalent to 10 mg of Orlistat was weighed and dissolved in 10 mL of methanol with shaking. The above drug solution was filtered through whatmann's filter paper No.41 to get concentration of 1 mg/mL solution. This was then diluted to make the working concentration of 100 µg/mL with methanol. From the above solutions suitable aliquots were prepared for method A and B respectively.

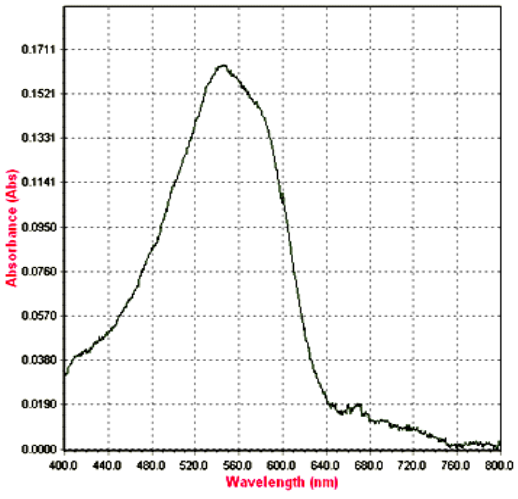

Fig 2: Visible Spectrum of Orlistat (Method A)

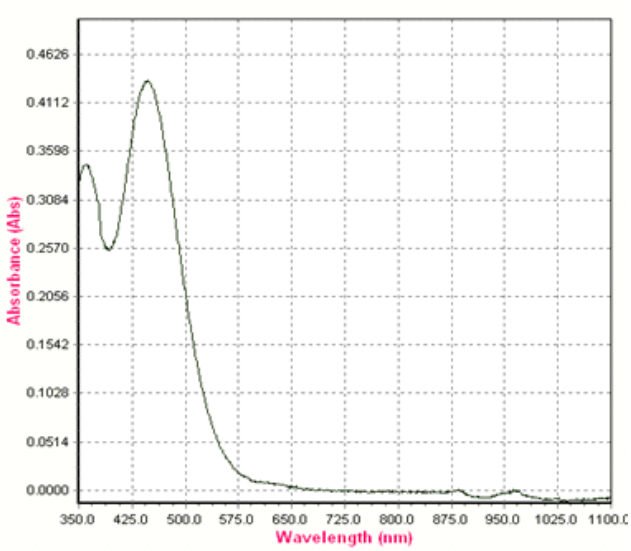

Fig 3: Visible Spectrum of Orlistat (Method B)

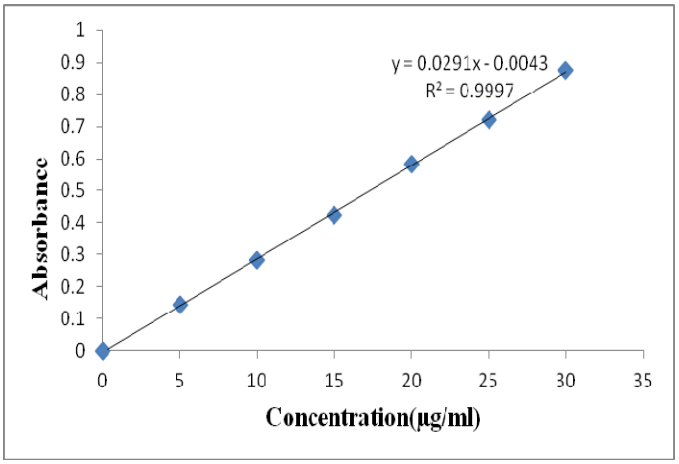

Fig.4. Calibration curve of Orlistat (Method A)

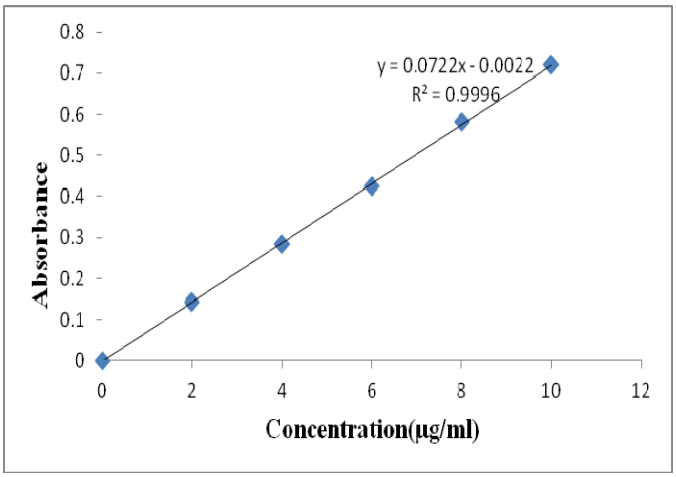

Fig.5. Calibration curve of Orlistat (Method B)

RESULTS AND DISCUSSION

Validation of analytical data [11]

The method was validated in accordance with the current ICH guidelines.

Linearity and range

The calibration graphs obtained by plotting the values of the absorbance versus the final concentrations (µg/mL) were found to be rectilinear over the concentration ranges of 5 - 30 µg/mL (r^2= 0.999) and 2 - 10 µg/mL (r^2=0.999) for method A and B respectively. The results indicate that the method was linear over the concentration range studied. The optical and regression parameters are summarized in **Table 1.**

Table 1: Optical Characteristics and Analytical Parameters of the Proposed Methods

Parameters	Method A	Method B
λ_{max} (nm)	546	443
Beer's law limits (µg/mL)	5 – 30	2 - 10
Molar absorptivity (lit mol^{-1} cm^{-1})	1.5×10^5	5.3×10^5
Limit of Detection (LOD/ µg/mL)	0.07	0.221
Limit of quantitation(LOQ/ µg/mL)	0.23	0.671
Sandell's sensitivity (µg/cm^2 /0.001 A.U.)	0.003258355	0.00093304
Regression equation (Y*)		
Slope (b)	0.0291	0.0772
Intercept (a)	0.0043	0.0022
Correlation coefficient (r^2)	0.999	0.999
% RSD**	0.59	0.8
% range of errors		
Confidence limits with 0.05 level	0.0486	0.0064
Confidence limits with 0.01 level	0.076	0.01

* Y= bX+a, where X is the concentration of Orlistat in µg/mL and Y is the absorbance at respective λ_{max}

** For six replicate samples.

Table 2: Intra-Day and Inter-Day Precision for Method A

Con. taken (µg/mL)	Intra-day		Inter-day	
	Con. found* (µg/mL)	%RSD	Con. found* (µg/mL)	%RSD
10	10.02	0.96	9.89	0.98
15	14.98	0.86	14.91	0.88
20	19.89	0.78	19.85	1.01

*average of six determinations

Table 3: Intra Day and Inter Day Precision for Method B

Con. taken (µg/mL)	Intra-day		Inter-day	
	Con. found* (µg/mL)	%RSD	Con. found* (µg/mL)	%RSD
4	3.98	0.94	3.95	0.96
6	5.93	0.86	5.91	0.91
8	7.95	0.96	7.94	1.2

*average of six determinations

Table 4: Recovery Studies for Method A

Level of recovery	Sample conc. (µg/mL)	Standard conc. added (µg/mL)	Total conc. (µg/mL)	Conc. recovered (µg/mL)	% Recovery	Mean % recovery±%RSD
80%	20	16	36	15.9	99.4	99.7±0.12
	20	16	36	16.09	100.6	
	20	16	36	15.88	99.3	
100%	20	20	40	20.2	101	100.3±0.35
	20	20	40	20.1	100.5	
	20	20	40	19.9	99.5	
120%	20	24	44	24.09	100.4	100.3±0.23
	20	24	44	23.88	99.5	
	20	24	44	24.28	101.2	

Table 5: Recovery Studies for Method B

Level of recovery	Sample Conc. (µg/mL)	Standard conc. added (µg/mL)	Total Conc. (µg/mL)	Amount Recovered (µg/mL)	% Recovery	Mean % Recovery±%RSD
80%	6	4.8	10.8	4.77	99.5	99.9±0.76
	6	4.8	10.8	4.8	100	
	6	4.8	10.8	4.81	100.4	
100%	6	6	12	5.94	99	100.1±0.43
	6	6	12	6.01	100.3	
	6	6	12	6.06	101	
120%	6	7.2	13.2	7.15	99.4	100.4±0.21
	6	7.2	13.2	7.31	101.6	
	6	7.2	13.2	7.21	100.2	

Table 6: **Estimation of Orlistat in Pharmaceutical Formulation**

Formulation	Labeled amount	Amount found		% Recovery	
		Method A	Method B	Method A	Method B
Capsule 1	60 mg	59.98 mg	59.76 mg	99.96	99.60
Capsule 2	120 mg	119.92 mg	119.89 mg	99.93	99.90

Precision

The precision of analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under the prescribed condition. The system precision was analysed by six different solutions of same concentration and absorbances were noted. The result was indicated by % RSD. The results are shown in **Table 1**. Repeatability or Intra-day precision was investigated on six replicate sample solutions on the same day. Inter-day precision was assessed by analyzing newly prepared sample solutions in triplicate over three consecutive days. Both inter day and intraday precision was expressed as % RSD. The % RSD values for intraday precision for Method A was 0.78-0.96 and for Method B 0.86-0.96. The % RSD for inter day precision for Method A and Method B are 0.88-1.01 and 0.91-1.2. The results were summarized in **Table 2 & 3**. The low value of % RSD for both methods indicates the high precision of the both methods.

Accuracy

Accuracy of the method was determined by preparing solutions of different concentrations that is 80%, 100% and 120% in which the amount of marketed formulation was kept constant and the amount of pure drug was varied. Solutions were prepared in triplicates and accuracy was indicated by % recovery. The results for both Method A and Method B were a shown in **Table 4** & **Table 5** respectively.

Limit of Quantitation (LOQ) and Limit of Detection(LOD)

LOD and LOQ were determined based on statistical calculation from the calibration curves, where LOD = (3.3 ×σ)/m; LOQ= (10.0×σ)/m (σ is the standard deviation of the y-intercepts of the three regression lines and m is mean of the slopes of the three calibration curves) .The LOD and LOQ values of the developed methods were summarized in **Table 1.**

Application of proposed method to formulation

The proposed methods are applied to pharmaceutical formulation and results are shown in **Table 6**.

CONCLUSION

The two visible spectrophotometric methods proposed for the determination of Orlistat, which are fairly sensitive, simple and economical with reasonable precision and accuracy. Parameters and statistical comparison justify this method for application in estimation of Orlistat in pure and dosage form. Moreover the methods are free from interference by common additives and excipients for the assay and evaluation of Orlistat in pharmaceutical dosage form.

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