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RESEARCH ARTICLE

UV Spectrophotometric Method Development and Validation for Determination of Cetilistat in API and in Pharmaceutical Dosage Form

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ABSTRACT

Simple, rapid, sensitive, precise and specific UV Spectrophotometric for the determination of cetilistat in API and pharmaceutical dosage form were developed and validated. In this method solutions of cetilistat were prepared in n-hexane. Cetilistat standard solution was scanned in the UV range (400-200nm) in a 1cm quartz cell in a double beam UV spectrophotometer. The standard solution of cetilistat showed maximum absorption at wavelength 320.0 nm. The method obeys Beer's law in the concentration range from 20-100µg/ml. The correlation coefficient was found to be 0.9996 and regression of the curve was found Y=0.0096x+0.0012 with excellent recovery 96-99%. Limit of detection and limit of quantification were found to be 2.7283µg/ml and 8.2677µg/ml respectively. The ruggedness and robustness were performed. The method was validated for several parameters like accuracy, precision as per ICH guidelines. Statistical analysis proved that the methods are repeatable and specific for determination of the said drug. These methods can be adopted in the routine assay analysis of cetilistat in API and pharmaceutical dosage form.

KEYWORDS

Cetilistat, UV Spectrophotometry, Absorbance maxima, Method validation

INTRODUCTION

Obesity is a growing public health problem, and in many countries, it has reached epidemic proportions. It is a complex multifactorial disorder characterized by an excess accumulation of adipose tissue, and is associated with increased risk for a number of complications, including type-2 diabetes, cardiovascular disease, hypertension, dyslipidemia and cancer. A sustained weight loss of between 5 and 10% of initial body weight has been associated with clinically meaningful reductions in these obesity-related comorbidities.

One approach is to induce a negative energy balance by targeting fat metabolism and gastrointestinal (GI) lipases in particular. Inhibition of lipases reduces hydrolysis of dietary triglycerides, limiting the absorption of monoglycerides and free fatty acids, thereby enhancing weight loss.

Orlistat (Xenical), an inhibitor of GI lipases approved for use in the long-term management of obesity and associated co-morbidities in adults and adolescents, can be associated with a number of undesirable GI-related adverse events. These include oily spotting, flatus with discharge, oily evacuation and faecal incontinence, which can reduce patient compliance and withdrawal from treatment.

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Cetilistat is a novel highly lipophilic benzoxazinone that inhibits GI and pancreatic lipases, which raises the possibility of a distinct clinical profile, and is in development for the management of obese patients with or without complications.

Cetilistat is a drug designed to treat obesity. It acts in the same way as the older drug orlistat (Xenical) by inhibiting pancreatic lipase, an enzyme that breaks down triglycerides in the intestine. Without this enzyme, triglycerides from the diet are prevented from being hydrolyzed into absorbable free fatty acids and are excreted undigested.[2,3,4]

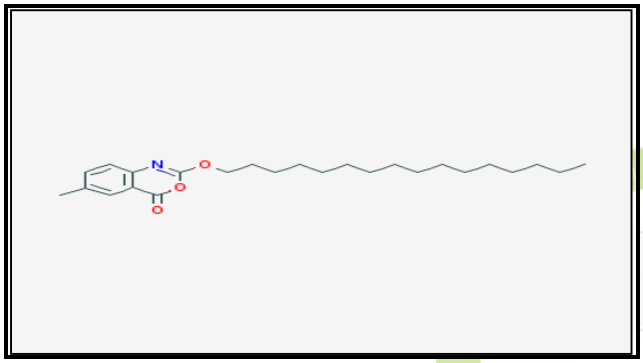

Figure 1: Chemical structure of Cetilistat[5]

Cetilistat, (2-hexadecoxy-6-methyl-3,1-benzoxazin-4-one). It is a white to off- white powder which is freely soluble in dichloromethane, chloroform, acetone, carbon tetra-chloride, chlorobenzene, n-hexane, diethyl ether.

Slightly soluble in ethyl acetate and toluene, and insoluble in methanol and water.

The present work is to Develop and Validate UV Spectrophometric Method for The Determination of Cetilistat in API and its Pharmaceutical Dosage Form.

MATERIAL & METHODS

Instruments

For weighing, a calibrated weighing balance (Shimadzu) of 1 mg sensitivity was used.

A Systronic UV-visible double beam spectrophotometer- 2201 was used.

All other glasswares and apparatus were made up of borosilicate and were calibrated.

Chemicals

API- Cetilistat is pure drug purchased from Swapnroop Drugs & Pharmaceuticals, Aurangabad, Maharashtra, India.

Tablets of 60 mg strength were purchased from the local pharmacy in Solapur under commercially available brand name Cetislim (Akums Drugs and Pharmaceuticals Ltd.), n-hexane 95% LR was used in this study.

UV Spectroscopic Method

Solvent Selection

Cetilistat was soluble in n-hexane. In the present investigation n-hexane was selected as a solvent.

Preparation of Standard Stock Solution

The standard stock solution of cetilistat was prepared by transferring, accurately weighed 10 mg of cetilistat to 10 ml of volumetric flask containing 5 ml n-hexane. Dissolve drug properly. Then volume was made up to the mark by using n-hexane to give concentration 1000 µg/ml. From this 2.5 ml of the solution was transferred to a 25 ml volumetric flask and make up the volume with n-hexane to give a concentration of 100 µg/ml which is a standard stock solution and it is further diluted with n-hexane to get concentration range of 20-100 µg/ml.

Determination of Absorption Maxima

The standard stock solution of 100µg/ml was scanned in the range of 400-200 nm to determine the wavelength of Maximum Absorption. The drug showed Absorption maxima at 320.0 nm.

Preparation of Calibration Curve

For the preparation of the calibration curve, the concentration of 20-100µg/ml were prepared by pipetting out 2, 4, 6, 8 and 10 ml of the 100 µg/ml solution into 10 ml volumetric flask and made up the volume with n-hexane. The absorbance of each solution was measured at 320.0 nm against n-hexane as a blank.

Calibration curve of the cetilistat was plotted by taking the absorbance obtained on the y-axis

and the concentration of the solution on the x-axis. The curve showed linearity in the range of 20-100 µg/ml with correlation coefficient 0.9996.

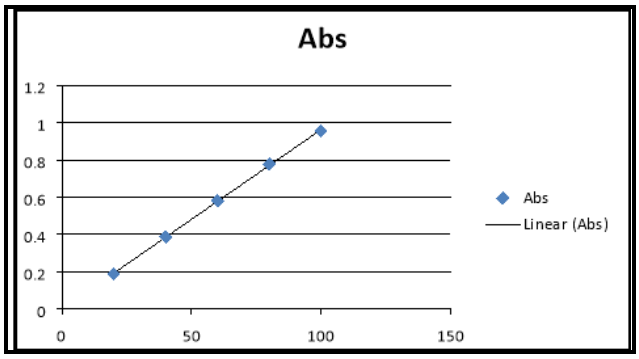

Figure 2: Calibration curve of Cetilistat

Quantitative Analysis of Pharmaceutical Tablet Dosage Form

Twenty tablets were weighed accurately and powdered. Powder equivalent to 10 mg cetilistat was weighed and transferred to a 10 ml volumetric flask. It was dissolved in 10 ml n-hexane and sonicate for 15 minutes to get a homogeneous solution.

Then it was filtered through a 0.45 µ Whatman filter paper. A final concentration of 100 µg/ml of cetilistat was prepared. This solution was filtered through filter paper to remove some un-dissolved excipients. After filtration, from this 6 ml was taken and diluted to 10 ml with n-hexane which gives 60 µg/ml solution and the absorbance of the solution was measured at 320.0 nm.

Table 1: Results obtained in the determination of cetilistat in tablet dosage form

Tablet Formulation	Label claim	Amount Taken	Amount found	Assay %
Cetislim	60 mg	60 µg/ml	60.66 µg/ml	101.1%

Method Validation

The developed method was validated as per ICH guidelines for following Parameters

1. ***Linearity:*** 2, 4, 6, 8, 10 ml of Standard solution was transferred into a series of 10 ml volumetric flasks. The volume was made up to the mark with n-hexane to obtain the concentration of 20, 40, 60, 80, 100 µg/ml. Then absorption of this solution was recorded and the graph was plotted of absorption against concentration. The correlation coefficient (r^2) of least square linear regression of cetilistat was calculated

2. **Range:** The Range of the analytical method was decided from the interval between upper and lower level of calibration curve by plotting curve.

3. **Accuracy:** Recovery study was carried out by the standard addition method by adding a known amount of cetilistat to the preanalyzed sample at three different concentration levels that is 80%, 100%, 120% of assay concentration and percent recovery were calculated.

   2 ml of tablet solution was transferred to 4 different 10 ml volumetric flasks (labelled as blank, 80%, 100%, 120%) separately and 0, 1.6, 2, 2.4 ml of 100 µg/ml standard solution was added respectively and the volume was made up to the mark with n-hexane. Absorbances were noted for these samples.

   Standard deviation and %RSD was calculated. Accuracy is reported as % recovery, which was calculated from the expression as equation given below,

   $$\% \text{ Recovery} = \frac{\text{Observed value}}{\text{True value}} \times 100$$

4. ***Precision:*** The precision of an analytical procedure expresses the closeness of agreement (degree of scattering) between a series of measurements obtained from multiple sampling of the same sample under the prescribed conditions. The precision of the method was determined in terms of repeatability and intraday and interday precisions.

   Intraday and interday precision (Intermediate Precision):

Intraday precision was determined by analyzing the drugs at concentrations (60µg/ml) and each concentration for three times, on the same day.

Interday precision was determined similarly, but the analysis being carried out daily, for two consecutive days.

Repeatability

Repeatability of the method was determined by analyzing six samples of same concentrations of the drug (60µg/ml). Absorbance of each was measured.

5. **Robustness:** The robustness of the developed method is its capacity to remain unaffected by small changes in altered conditions. To determine the robustness of the method, the wavelength of analysis was deliberate and the assay was evaluated. The effect of detection wavelength was studied at ±5 nm.
6. **Ruggedness:** Ruggedness was determined by carrying out analysis by two different analysts and the respective absorbance was noted and the results were indicated as % RSD.
7. **Limit of Detection**: Detection limit was determined based on the standard deviation of absorbance of same concentration that is a standard solution of cetilistat (60µg/ml) prepared six times and LOD calculated by

$$LOD = 3.3(SD/S)$$

Where, SD- standard deviation; S= slope of the curve

8. ***Limit of Quantification:*** Quantification limit was determined based on the standard deviation of peak area of same concentration that is standard solution cetilistat (60µg/ml) prepared six times and LOQ calculated by

$$LOD = 10(SD/S)$$

Where, SD= standard deviation; S= slope of Curve

RESULTS

Determination of wavelength of maximum absorption

The wavelength of maximum absorption was found to be 320.0 nm.

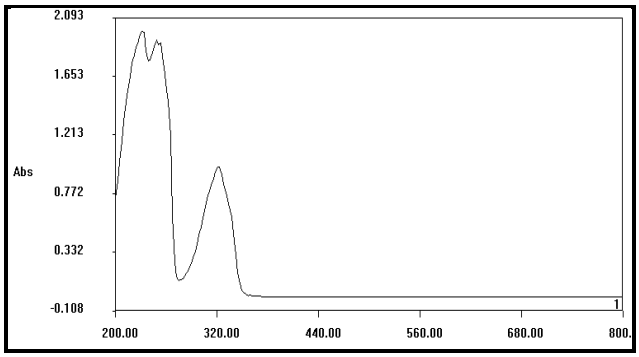

Figure 3: Wavelength of maximum absorption of cetilistat

Linearity

The linearity of this method was determined at ranges from 20-100 µg/ml for cetilistat.

The regression equation was found to be Y=0.0096x +0.0012 be, r^2=0.9996.

Table 2: Linearity table

Sr. No	Concentration (µg/ml)	Absorbance
1	20	0.189
2	40	0.387
4	60	0.581
4	80	0.777
5	100	0.955

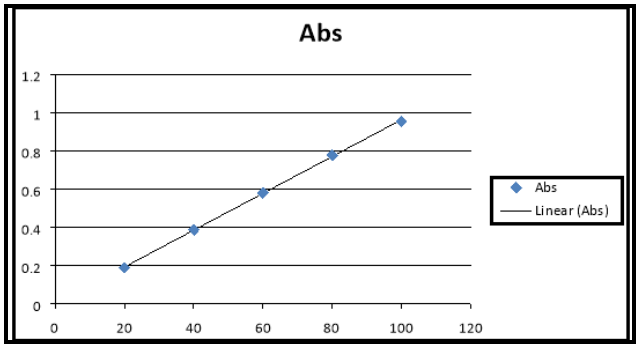

Figure 4: Linearity graph of cetilistat

The linearity for cetilistat was found to be linear in the range of 20-100µg/ml with r^2= 0.9996 and the straight line equation as Y= 0.0096x+0.0012.

Accuracy

The accuracy of the analytical method for cetilistat was determined at 80%, 100% and 120% levels of standard solution. Absorbance was measured at 320.0 nm and results were expressed in terms of % recoveries.

Table 3: Table for accuracy

Sr. No	Level of % Recovery	Amount of Tablet sample (ml)	Amt of std drug added (µg/ml)	Amt added µg	Amt found (µg/ml)	% Recovery
1	0	2	0	0	0	-
2	80	2	1.6	36	34.66	96.29
3	100	2	2	40	39.11	97.77
4	120	2	2.4	44	43.55	98.98

Precision

The precision (measurement of intraday, interday, repeatability) results showed good reproducibility with the present relative standard deviation (% RSD) was below 2.0% .

This indicated that method was highly precise.

Intraday precision

Table 4: Intraday morning precision

Sr. no	Conc (µg/ml)	Absorbance	SD	% RSD
1	60	0.597		
2	60	0.580		
3	60	0.582	0.007441	1.26
4	60	0.594		
5	60	0.580		
6	60	0.584		
		ȳ=0.586		

Table 5: Intraday afternoon precision

Sr. no	Conc. (µg/ml)	Absorbance	SD	% RSD
1	60	0.598		
2	60	0.600		
3	60	0.612	0.00776	1.191
4	60	0.595		
5	60	0.599		
6	60	0.611		
		ȳ =0.602		

Table 6: Intraday evening precision

Sr. no	Conc. (µg/ml)	Absorbance	SD	% RSD
1	60	0.592		
2	60	0.593		
3	60	0.598	0.007026	1.174
4	60	0.595		
5	60	0.608		
6	60	0.607		
		ȳ =0.598		

Interday Precision

Table 7: Interday morning precision study

Sr. no	Conc. (µg/ml)	Absorbance	SD	% RSD
1	60	0.618		
2	60	0.629		
3	60	0.643		
4	60	0.627	0.009304	1.472
5	60	0.633		
6	60	0.641		
		ȳ =0.631		

Table 8: Interday afternoon precision study

Sr. no	Conc. (µg/ml)	Absorbance	SD	% RSD
1	60	0.621		
2	60	0.622		
3	60	0.633	0.006616	1.053
4	60	0.625		
5	60	0.628		
6	60	0.638		
		ȳ =0.627		

Table 9: Interday evening precision study

Sr. no	Conc. (µg/ml)	Absorbance	SD	% RSD
1	60	0.633		
2	60	0.651		
3	60	0.648		
4	60	0.637	0.011725	1.81
5	60	0.643		
6	60	0.666		
		ȳ =0.646		

Repeatability

Table 10: Repeatability study

Sr. no	Conc. (µg/ml)	Absorbance	SD	% RSD
1	60	0.597		
2	60	0.580		
3	60	0.582	0.007441	1.269
4	60	0.594		
5	60	0.580		
6	60	0.584		
		ȳ =0.586		

Limit of Detection

Table 11: For Limit of Detection

LOD (µg/ml)	2.7283 µg/ml

Limit of Quantification

Table 12: For Limit of Quantification

LOQ (µg/ml)	8.2677 µg/ml

Robustness

Table 13: Robustness study

Sr. No	Wavelength (nm)	Absorbance	SD	% RSD
1	315	0.559		

2	316	0.572		
3	317	0.587		
4	318	0.612		
5	319	0.619	0.022137	3.68
6	320	0.622		
7	321	0.620		
8	322	0.619		
9	323	0.612		
10	324	0.604		
11	325	0.581		
		$\bar{y}$ =0.600		

Ruggedness

Ruggedness was determined by carrying out analysis by two different analysts and the respective absorbance was noted and the results were indicated as % RSD.

Table 14: For Ruggedness

Analyst-1		
Concentration (µg/ml)	Absorbance	Statistical analysis
60	0.677	
60	0.677	Mean=0.677
60	0.678	SD=0.000516
60	0.678	%RSD=0.0762186
60	0.677	
60	0.677	
Analyst-2		
60	0.670	
60	0.670	Mean=0.669
60	0.669	SD=0.000548
60	0.669	%RSD=0.0819133
60	0.670	
60	0.669	

Preliminary Analysis of Cetilistat

Preliminary analysis of cetilistat such as description, solubility was performed.

UV-Spctrophotometry for Cetilistat

Cetilistat being UV absorbing has been successfully employed for its quantitative determination by UV Spectrophotometric method. Being soluble in n-hexane, stock solutions and working standards were made in n-hexane. The maximum wavelength of absorption of drug was determined by taking scan of the drug solution in the UV region (200-400 nm).the correlation of the standard curve for the drug was 0.9996. The accuracy was from 96-99% at 320 nm.

The proposed method showed absorption maxima at 320 nm and obeyed Beer's law in the concentration of 20-100 µg/ml. The limit of detection (LOD) was found to be 2.7283 µg/ml and limit of quantification (LOQ) to be 8.2677 µg/ml respectively. All statistical data prove the validity of the proposed method, which can be applied for routine analysis of cetilistat.

Assay of Tablet Formulation

Amount of drug present in tablet formulation was calculated using equation at 320 nm, and Y=0.0096x+0.0012 and the amount of cetilistat was found to be 101.1% of label claim respectively.

This method can be employed for routine analysis of cetilistat.

SUMMARY AND CONCLUSION

Summary of UV Spectrophotometeric Method of Cetilistat

Table 15: For Summary

Sr. No	Parameters	Values
1	Beer's law limit (µg/ml)	20-100
2	Absorption maxima (nm)	320.0

3	Standard Regression Equation	Y=0.0096x +0.012
4	Correlation Coefficient (r^2)	0.9996
5	Accuracy	96-99%
6	Precision (%RSD) Repeatability	1.269%
7	LOD	2.7283 µg/ml
8	LOQ	8.2677µg/ml
9	Robustness (%RSD)	3.68%
10	Ruggedness(%RSD)	0.0762186 and 0.0819133
11	Assay (%)	101.1%

CONCLUSIONS

The UV-spectrophotometric method was developed and it is found to be simple, accurate, precise, highly sensitive, reproducible and inexpensive. The proposed method was found suitable for determination of cetilistat in API and its pharmaceutical dosage form without any interference from the excipients. This method can be effectively applied to the routine analysis of cetilistat in API. Its advantages are the low cost of reagents, speed and simplicity of sample treatment, satisfactory precision and accuracy.

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