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# DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD OF DRUG CETILISTAT IN BULK AND PHARMACEUTICAL FORMULATION

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## ABSTRACT

Cetilistat is a new drug utilized in a treatment of obesity. Since no High Performance Liquid Chromatographic method has been reported in literature, an attempt was made to develop and validate a simple, rapid accurate and precise stability indicating RP-HPLC method for estimation of cetilistat in bulk and pharmaceutical dosage forms as per ICH guidelines. The separation was carried out by using mobile phase consisting of acetonitrile and phosphate buffer of pH 4.0 in the ratio 60:40 using BDS Hypersil C-18 (250×4.6µm) at the flow rate of 1.0 ml/min. The retention time of cetilistat was found to be 2.73 min at wavelength 228 nm. The method was found to be linear over the concentration range of 20-100 µg/ml with correlation coefficient 0.9986. Mean percent recovery of cetilistat sample solutions was found to be 100.26%. The limit of detection and limit of quantification for cetilistat was found to be 1.961µg/ml and 5.944 µg/ml respectively. To check the stability of drug, forced degradation was carried out under different stress condition recommended by International Conference on Harmonization (ICH). In acidic condition mild degradation was seen whereas significant degradation took place under alkaline and thermal condition. No degradation was seen under oxidative condition. From the results it was found that the proposed RP-HPLC method was simple, rapid, precise, accurate, robust and stability indicating and hence can be successfully used for the routine analysis of cetilistat in pharmaceutical formulation.

**KEYWORDS:** Cetilistat, Reverse Phase HPLC, Method validation, Forced degradation.

## INTRODUCTION

Cetilistat, chemically is a (2-hexadecoxy-6-methyl-3, 1-benzoxazin-4-one) (Figure.1). It is used in a treatment of obesity. It acts by inhibiting the enzyme pancreatic lipase that breaks down triglycerides in the intestine. It is a benzoxazinone derivative which is highly lipophilic that inhibits GI and pancreatic lipases that blocks fat digestion and absorption leading to reduce energy intake and thereby weight loss.[1-6]

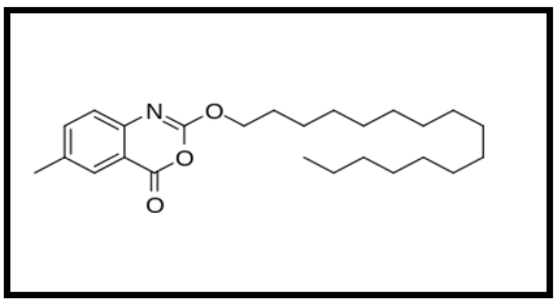


**Figure 1: chemical structure of Cetilistat.**

The literature survey reveals that only one spectrophotometric method has been reported for cetilistat.[7] No chromatographic method was reported for cetilistat till date. Thus the aim of the present work is to develop and validate stability indicating RP-HPLC

method for determination of cetilistat in API and its pharmaceutical dosage form.

## MATERIALS AND METHODS

### Chemicals and Reagents

Analytical and HPLC grade water, acetonitrile, methanol were used for the study. AR grade disodium hydrogen phosphate and potassium hydrogen phosphate were used. Cetilistat is pure drug purchased from Dhamtec Pharma and Consultants Navi Mumbai, Maharashtra, India. Cetilistat tablets of 60 mg were purchased from local pharmacy under commercially available brand name Kilfat (Akumentis Healthcare Ltd).

### Instruments

HPLC-Jasco (Model/PU20801/UV-2075-Plus, Jasco) with Borwin software and BDS Hypersil $C_{18}$ column (250×4.6mm) with UV- Visible detector was used for analysis.

### HPLC Method

### Solvent Selection

Cetilistat was soluble in acetonitrile. In the present study acetonitrile was considered as a solvent with small amount of DMSO to enhance the solubility.

### Selection of wavelength

10mg of Cetilistat was weighed accurately and transferred to 10ml volumetric flask and volume was made up to mark with acetonitrile and small amount of DMSO to give 1000µg/ml of solution. From this 1ml was pipetted out and was transferred into 10ml of volumetric flask and volume was made up to mark with acetonitrile to give 100µg/ml of solution. The above solution was scanned in the range of 400.0 nm to 200.0 nm using UV-Vis spectrophotometer using acetonitrile as a blank. Maximum absorbance was shown at 228nm as shown in Fig. 2. Hence the same wavelength was selected for analysis of Cetilistat.

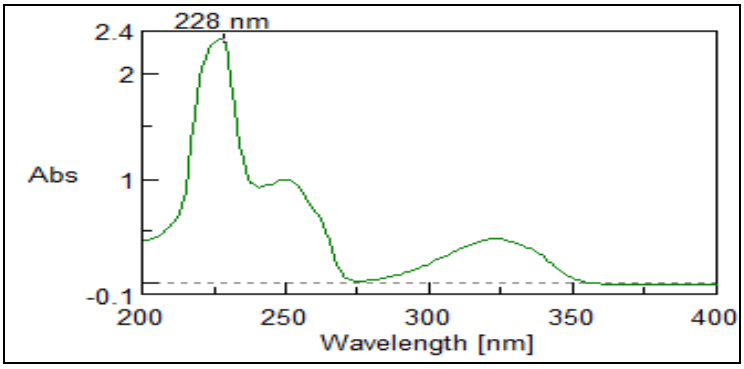


**Figure 2: UV Spectra of Cetilistat.**

### Preparation of Buffer for mobile phase

Phosphate buffer pH 4.0 was prepared by dissolving 0.504g of disodium hydrogen phosphate and 0.301g of potassium dihydrogen phosphate in sufficient water to produce 100ml. Further the pH was adjusted with glacial acetic acid.

### Preparation of Mobile Phase

Phosphate buffer pH 4.0 and acetonitrile in the ratio of 60:40% v/v was used as mobile phase for the present study. The mobile phase was sonicated and degassed using ultra sonicator.

### Preparation of Standard solution

The standard stock solution of cetilistat was prepared by transferring, accurately weighed 10 mg of cetilistat to 10 ml of volumetric flask containing 1ml of DMSO, 6 ml of acetonitrile. Then volume was made up to the mark by using acetonitrile to give concentration 1000 µg/ml, and then it was sonicated for 10mins.From this 2.5 ml of the solution was transferred to a 25 ml volumetric flask and make up the volume with mobile phase to give a concentration of 100 µg/ml which is a standard stock solution and it is further diluted with acetonitrile to get concentration range of 20-100 µg/ml.

### Preparation of sample stock solution

Twenty tablets were weighed accurately and powdered. Cetilistat equivalent to 10 mg was weighed and transferred to a 10 ml volumetric flask containing 1ml DMSO and 9 ml acetonitrile and was sonicated for 15 minutes to get a homogeneous solution. 100 µg/ml concentration of cetilistat was prepared and was used as stock solution. This solution was filtered through 0.45µm filter before injecting into HPLC system.

### Method Validation[8-13]

Validation of RP-HPLC method was done as per ICH guidelines for parameters like linearity, accuracy, precision, robustness, LOD and LOQ.

### System suitability

System suitability parameters were assessed by preparing standard solutions of Cetilistat. The solutions were injected six times at a concentration range of 60 µg/ml and various parameters like retention time, theoretical plates, tailing factor and peak area were calculated.

### Specificity

Solutions of standard and sample were prepared and injected in to HPLC system and its respective peak area and retention time were observed.

**Linearity**
Suitable quantity of standard solution was transferred into a series of 10 ml volumetric flasks. The volume was made up to the mark with mobile phase to obtain the concentration of 20, 40, 60, 80, 100 µg/ml. Peak area of this solution was recorded and the graph was plotted against concentration. The correlation coefficient ($R^2$) of least square linear regression of cetilistat was calculated.

**Accuracy**
Recovery studies were determined by standard addition methods by adding known amounts of cetilistat to pre-analyzed samples at three different concentration levels i.e. 80%, 100%, 120% of assay concentration and percent recovery was calculated. 3 ml of tablet solution was transferred to 3 different 10 ml volumetric flasks separately and 2.4, 3, 3.6 ml of 100 µg/ml standard solution was added to the flask and the volume was made up to the 10 ml with mobile phase. Peak area, standard deviation and % RSD was calculated.

**Precision**
The precision of the method was determined in terms of repeatability and intraday and interday precisions. Intraday and Interday precision was determined by analyzing the drugs in triplicate at concentrations 40, 60 and 80 µg/ml.

**Assay**
The drug content in sample and standard was found to be comparable with no interference of excipients in it. For the study 20 tablets were weighed and its average weight was calculated. Cetilistat equivalent to 10 mg was weighed and transferred to a 10 ml volumetric flask containing 1ml DMSO and 9 ml acetonitrile and was sonicated for 15 minutes to get a homogeneous solution. 100 µg/ml concentration of cetilistat was prepared and was filtered through 0.45µm filter. After filtration 6 ml was taken and diluted to 10 ml of acetonitrile to give 60 µg/ml of solution. The resultant solution was injected in to HPLC system and peak area was calculated.

**Limit of Detection and Limit of Quantification**
Detection limit was determined based on the standard deviation of peak area and was calculated by formula LOD = 3.3(Standard deviation/Slope). Also Quantification limit was determined based on the standard deviation of peak area and was calculated by formula LOQ = 10(Standard deviation/Slope).

**Robustness**
Few parameters were deliberately varied for study of robustness. The Robustness was carried out by changing flow rate, wavelength and mobile phase composition. Flow rate, wavelength and mobile phase composition were varied by ±2% and the %RSD was calculated.

**Force Degradation Studies[14-19]**
Force degradation studies were carried out by treating the sample under the following conditions;

**Acid degradation**
To 4 ml of stock solution 1 ml of 0.01N HCL was added in the volumetric flask and it was kept at room temperature for 15 min. The solution was neutralized with 0.01 N NaOH and volume was made up to 10 ml with diluent. The resulting solution was sonicated and then injected in to HPLC system and the chromatogram was recorded.

**Alkali degradation**
To 4 ml of stock solution 1 ml of 0.01N NaOH was added in the volumetric flask and it was kept at room temperature for 15 min and then neutralized with 0.01 N HCL and volume was made up to 10 ml with diluent. The resulting solution was sonicated and then injected in to HPLC system and the chromatogram was recorded.

**Oxidative degradation**
To 4 ml of stock solution 3% of $H_2O_2$ was added in the volumetric flask and it was kept at room temperature for 1, 3 and 5 days and after that volume was made up to 10 ml with diluent. The resulting solution was sonicated and then injected in to HPLC system and the chromatograms were recorded on day 1, 3 and 5.

**Photolytic degradation**
The drug was exposed to UV radiation at 254 nm for 24 hrs. The exposed material was then placed in a volumetric flask and the mobile phase was added. The resulting solution was sonicated and then injected in to HPLC system. The chromatogram was recorded.

**Thermal degradation**
4ml of stock solution was kept in hot air oven at 70°C for 1hr and then cooled. Then the volume was made up to 10 ml with diluent. The resulting solution was sonicated and then injected in to HPLC system. The chromatogram was recorded.

## RESULT AND DISCUSSION

**HPLC Method Optimization**
For method optimization various mobile phases were tried in different ratios, such as acetonitrile: water (60:40) v/v, acetonitrile: methanol: water (50:25:25v/v/v), acetonitrile: phosphate buffer pH 3.2 (60:40v/v) etc. All these mobile phases were unacceptable due to tailing, fronting and no sharpness in the peak. After various trials mobile phase consisting of acetonitrile: phosphate buffer pH 4.0 (60:40v/v) was selected which gave sharp peaks with no tailing and fronting. The chromatogram of standard cetilistat was shown in Fig 3. Optimized chromatographic conditions and system suitability parameter were shown in Table 1.

**Table 1: Optimized chromatographic conditions and system suitability parameter.**

| | |
|---|---|
| Column | BDS Hypersil C18 |
| Wavelength | 228 nm |
| Column Temperature | Ambient |
| Injection Volume | 10 µl |
| Run time | 10 min |
| Flow rate | 1.0 ml / min |
| Pump mode | Isocratic |
| Mobile phase | Acetonitrile : phosphate buffer 4.0 (60:40v/v) |
| Retention time | 2.25 minutes |
| Concentration | 50ppm |
| Tailing factor | 1.476 |
| Theoretical plates | 2586 |
| Peak area | 484745 |

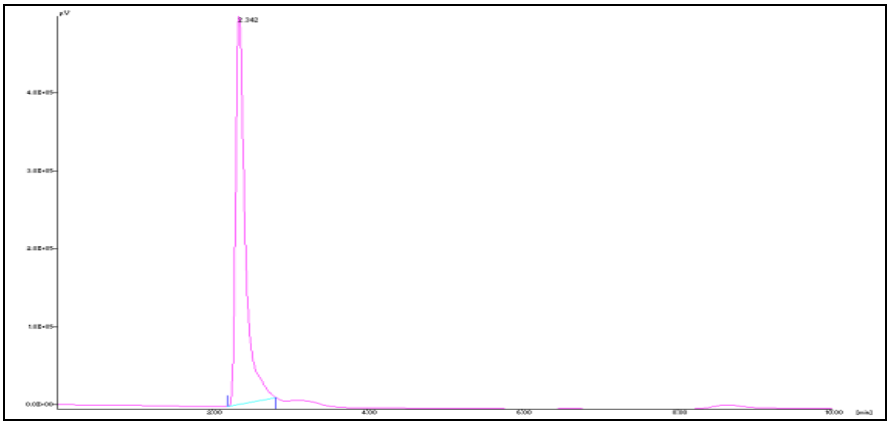

**Figure 3: Typical chromatogram of standard solution.**

**HPLC Method Validation**

**Linearity**

The linearity for cetilistat was determined in the range of 20-100µg/ml. The regression equation was found to be Y=47209x+2E+06 and $r^2$=0.9986. Data for calibration curve was shown in Table 2 and the calibration curve was shown in Fig 4.

**Table 2: Calibration data of cetilistat.**

| Sr no | Concentration (µg/ml) | Peak area |
|---|---|---|
| 1 | 20 | 3134867 |
| 2 | 40 | 4004667 |
| 3 | 60 | 4939683 |
| 4 | 80 | 5856974 |
| 5 | 100 | 6929654 |

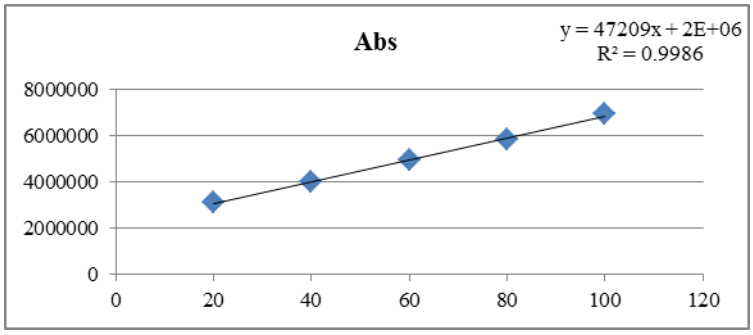


**Figure 4: linearity graph of Cetilistat.**

**LOQ and LOD**

LOD and LOQ were calculated from the equation and were found to be 1.961µg/ml and 5.944 µg/ml respectively shown in Table 3.

**Table 3: LOD and LOQ of cetilistat.**

| Sr no | Parameter | Result |
|---|---|---|
| 1 | LOD | 1.961µg/ml |
| 2 | LOQ | 5.944 µg/ml |

**Accuracy**

The accuracy of the analytical procedure for cetilistat was determined at 80%, 100% and 120% levels of standard solution. Results were expressed in terms of % recoveries. The % recovery was found in the range from 99.96% to 100.57% and was shown in Table 4.

**Table 4: Recovery Studies (n=3)**

| Sr no | Level | Amount of sample (µg/ml) | Amount of Standard drug added(µg/ml) | Amount added (µg/ml) | Average peak area | Amount found (µg/ml) | % Recovery |
|---|---|---|---|---|---|---|---|
| 1 | 80 | 30 | 24 | 54 | 4548315 | 53.97 | 99.96% |
| 2 | 100 | 30 | 30 | 60 | 4840078 | 60.15 | 100.26% |
| 3 | 120 | 30 | 36 | 66 | 5133627 | 66.37 | 100.57% |

**Precision**

The precision results (measurement of intraday, interday, repeatability) showed good reproducibility and %RSD values were within limits which proved that method was highly precise. The results were shown in Table 5 and 6.

**Table 5: Interday Precision Studies (n=3)**

| Sr no | Conc. (µg/ml) | mean area | SD | %RSD |
|---|---|---|---|---|
| 1 | 40 | 4093781 | 71982.97 | 1.758349 |
| 2 | 60 | 4946999 | 42445.88 | 0.858013 |
| 2 | 80 | 5934314 | 70445.16 | 1.187082 |

**Table 6: Intraday Precision studies (n=3)**

| Sr no | Concentration (µg/ml) | mean area | Standard deviation | %RSD |
|---|---|---|---|---|
| 1 | 40 | 4037115 | 41861.29 | 1.036911 |
| 2 | 60 | 4975858 | 28062.86 | 0.56398 |
| 3 | 80 | 5858928 | 39749.02 | 0.678435 |

**Assay**

The drug content in the marketed formulation was found to be 100.26%. There was no interference of excipients in the marketed formulation. Results obtained were shown in table 7.

**Table 7: Assay results of pharmaceutical dosage forms.**

| Tablet formulation | Label claim | Amount taken | Amount found | % Assay |
|---|---|---|---|---|
| Kilfat | 60mg | 60µg/ml | 60.15µg/ml | 100.26 |

**Specificity**

Table 8 shows that retention time for standard and commercial product of cetilistat are same. This shows that, excipients do not interfere with the drug which proves the method is highly selective.

**Table 8: Specificity Studies of Cetilistat.**

| Sample name | Cetilistat area | Retention time |
|---|---|---|
| Standard | 4946999 | 2.25 |
| Sample | 4840078 | 2.28 |

**Robustness**

Robustness was carried out by deliberate modification of analytical parameters, which indicated that retention time and peak area remained unaffected by small changes in wavelength, mobile phase composition and flow rate. %RSD calculated was with in ICH limit thus indicating that method was sufficiently robust. The results were shown in Table 9.

**Table 2: Robustness results of Cetilistat.**

| Parameter | Change in parameter | % Estimation | Mean | S.D | %RSD |
|---|---|---|---|---|---|
| Wavelength (± 2nm) | 226 | 99.87 | 100.32 | 0.488 | 0.486 |
| | 228 | 100.26 | | | |
| | 230 | 100.84 | | | |
| Mobile phase composition (± 2%) | 58:42 | 100.25 | 100.39 | 0.245 | 0.244 |
| | 60:40 | 100.26 | | | |
| | 62:38 | 100.68 | | | |
| Flow rate ± (2%) | 0.8 | 99.75 | 100.12 | 0.327 | 0.326 |
| | 1.00 | 100.26 | | | |
| | 1.2 | 100.36 | | | |

**Force Degradation Study**

Chromatogram of acidic degradation showed mild degradation, whereas significant degradation took place under alkaline and thermal condition. Moderate degradation was observed when drug was subjected to UV light (254nm) for 24 hrs. No degradation took place under oxidative condition. Detailed study of degradation is shown in table 10.

**Table 30: Forced degradation study of cetilistat.**

| Sr No | Degradation | Condition | Duration | % Degradation |
|---|---|---|---|---|
| 1 | Acid degradation | 1ml of 0.01N HCl at room temperature | 15 min | 5.38 |
| 2 | Alkali degradation | 1ml of 0.01N NaOH at room temperature | 15 min | 18.66 |
| 3 | Oxidative degradation | 1ml 3% H2O2 at room temperature | 1,3 and 5 days | No degradation |
| 4 | Thermal degradation | 2ml solution in hot air oven at $70^0$ C | 1 hr. | 18.94 |
| 5 | Photolytic degradation | UV lamp(254nm) | 24hr | 3.97 |

## CONCLUSION

The proposed RP-HPLC method was successfully validated for parameters such as linearity, specificity, accuracy, precision, LOD, LOQ and robustness as per ICH guidelines. The method was found to be simple, accurate, precise, highly sensitive, reproducible and. The proposed method was found suitable for determination of cetilistat in API and its pharmaceutical dosage form with none interference from the excipients. All the validation parameters were within the acceptance limits. Hence this method can be effectively applied to the routine analysis of cetilistat in API. During forced degradation investigations, the degradation percentage of drug was with in limit as per ICH guidelines, confirming the method's stability indicating nature. Simple reagents and minimal preparation processes are used in this method for determining stability. Thus this technology can be utilised for routine analysis and quality control of cetilistat in pharmaceutical dosage form.

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## CONFLICT OF INTEREST

There is no conflict of interest regarding the publication of this article.

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