*ejpmr, 2022,9(8), 416-420*

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

# ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF CETILISTAT BY HPTLC METHOD

**Kanchan Chauhan* and Samie Sable**

Department of Quality Assurance, M.C.E. Society's Allana College of Pharmacy, 2390- B. K. B. Hidayatullah Road, Azam Campus, Camp, Pune, Maharashtra 411001.

***Corresponding Author: Kanchan Chauhan**
Department of Quality Assurance, M.C.E. Society's Allana College of Pharmacy, 2390- B. K. B. Hidayatullah Road Azam Campus, Camp, Pune, Maharashtra 411001.

**Article Received on 08/06/2022** **Article Revised on 28/06/2022** **Article Accepted on 18/07/2022**

## ABSTRACT

Drug cetilistat is used in the treatment of obesity. There is no high performance thin layer chromatographic method has been reported in literature till date. Thus an attempt was made to develop and validate a simple, rapid accurate and precise high performance thin layer chromatographic method for estimation of cetilistat in pharmaceutical dosage forms as per ICH guidelines. Cetilistat was chromatographed on silica gel $60F_{254}$ TLC plate using n-hexane and methanol as mobile phase in the ratio of 8:2 v/v. Cetilistat was quantified by densitometric analysis at 228 nm. The method was found to give compact spots for the drug with $R_f$ value 0.62± 0.01. The method was found to be linear over the concentration range of 200-1000 ng/spot with correlation coefficient 0.9985. The limit of detection and limit of quantification for Cetilistat was found to be 0.9094 ng/spot and 2.7558 ng/spot respectively. This method was validated with reference to linearity, accuracy, precision, specificity and robustness according to ICH guidelines.

**KEYWORDS:** Cetilistat, HPTLC, densitometric analysis, Method validation.

## INTRODUCTION

Drug cetilistat is a benzoxazinone derivative. Chemically it is 2-hexadecoxy-6-methyl-3, 1- benzoxazin-4-one (Fig.1). The drug is highly lipophilic that inhibits GI and pancreatic lipases that blocks fat digestion and absorption leading to reduce energy intake and thereby weight loss. It acts peripherally distinct to other anti-obesity agents which act on brain to reduce appetite.[1-6]

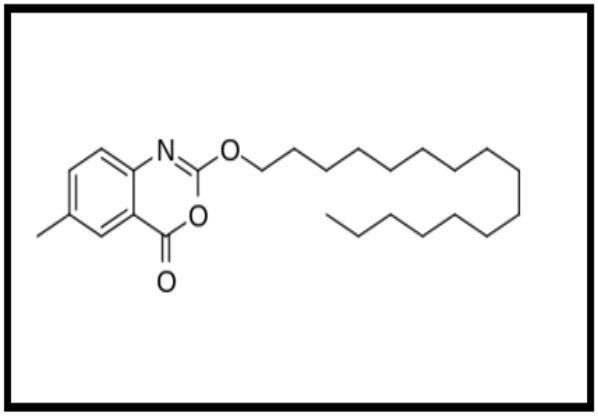


**Fig. 1: Structure of cetilistat.**

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

## MATERIALS AND METHODS

### Chemicals and Reagents

HPLC grade water, methanol were used for the study. API- Cetilistat is pure drug purchased from Dhamtec Pharma and Consultants Navi Mumbai, Maharashtra, India. Tablets of 60 mg were purchased from local

pharmacy in Pune under commercially available brand name Kilfat (Akumentis Healthcare Ltd).

**HPTLC instrumentation**

The sample solutions were applied in the form of bands of 6 mm width with a Hamilton syringe of 100 µl using a Camag Linomat 5 sample applicator on a pre-washed and activated precoated silica gel aluminium HPTLC plate $60F_{254}$ (6 cm x 10 cm with 250 m thickness). The slit dimension was fixed to 5 mm x 0.45 mm, and the scanning speed was set to 10 mm/s. Twin trough chamber were used for chamber saturation. The chamber saturation time for mobile phase was 20 min. Densitometric scanning was performed at 228 nm using CAMAG TLC Scanner 3 equipped with Wincats software 1.3.0.

**Solvent selection**

Cetilistat was freely soluble in n-hexane. Thus in the present study n-hexane was considered as a solvent for preparation of stock solution.

**Selection of wavelength**

10 mg of Cetilistat was weighed accurately and transferred to 10 ml volumetric flask and volume was made up to mark with n-hexane to give 1000 µg/ml of solution. From this 1ml was pipetted out and was transferred into 10 ml of volumetric flask and volume was made up to mark with n-hexane to give 100 µg/ml of solution. The above solution was scanned in the range of 200.0 nm to 400.0 nm using UV- Vis spectrophotometer using n-hexane as a blank.

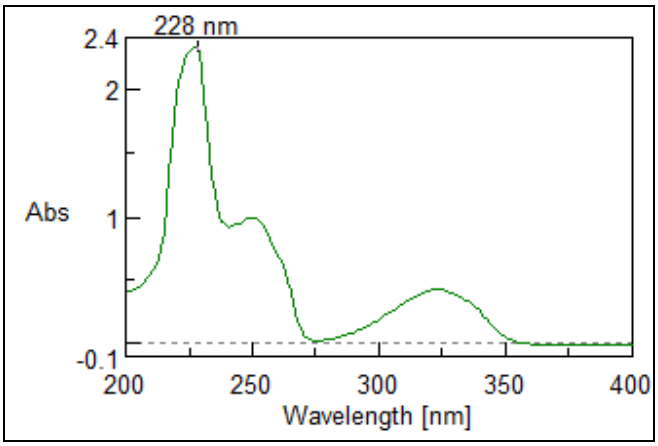


**Fig. 2: Absorbance spectra of cetilistat.**

**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 6ml of n-hexane. Then volume was made up to the mark by using n-hexane to give concentration 1000 µg/ml, and then it was sonicated for 10 mins. 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 get a concentration of 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 10 ml of n-hexane 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.[18-19]

**Method validation**

Validation of RP-HPLC method was done as per ICH guidelines for parameters like linearity, accuracy, precision, robustness, LOD and LOQ.[20]

**Specificity**

Solutions of standard and sample were prepared. It was observed that other substances present in the formulation did not interfere with the peak of cetilistat and thus the method was specific. The peak purity of cetilistat was checked by comparing the spectra at different level viz. peak start, peak apex and peak end position of the spot.

**Linearity**

Suitable quantity of standard solution was transferred into a series of 10ml volumetric flasks. The volume was made up to the mark with n-hexane to obtain the concentration of 200, 400, 600, 800, 1000 ng/spot. Peak areas and $R_f$ values of this solution were calculated 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. The percent recovery, 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 400,600 and 800 ng/band.

**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 wavelength, change in saturation time and change mobile phase volume. All these parameters were varied by ±2% and mean, S.D and % RSD were calculated.

**RESULT AND DISCUSSION**

Numerous initial trials were carried out with toluene, methanol, ethyl acetate, chloroform and n-hexane to determine their effect on drug migration. After these trials, various combinations of solvents, n hexane: acetonitrile (2.5:2.5), n-hexane: methanol (2.5:2.5), n-hexane: ethyl acetate (4:1), Dichloromethane: n-hexane (2.5:2.5), Acetonitrile: ethyl acetate: ammonia solution (2:2:1), Chloroform: acetonitrile: diethyl amine (2:2:1), n-hexane: methanol: ethyl acetate (2:2:1).

Better results were obtained in n hexane: methanol (4:1), where sharp peaks were observed with an $R_f$ of 0.63. Chromatogram of standard cetilistat is shown figure 3.

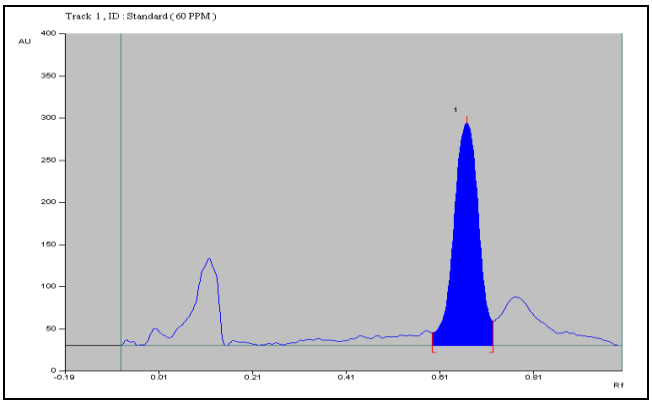


**Fig. 3: Densitogram of cetilistat.**

**Linearity**

The linearity for cetilistat was determined in the range of 200-1000 ng/spot. The regression equation was determined and the $R^2$ value was found to be 0.9985. Data for calibration curve was shown in Table 1 and the calibration curve was shown in figure 4.

**Table 1: Linearity range of cetilistat.**

| Sr. no. | Concentration (ng/spot) | Area | Rf |
|---|---|---|---|
| 1 | 200 | 3565.9 | 0.63 |
| 2 | 400 | 6720.9 | 0.62 |
| 3 | 600 | 10010.4 | 0.63 |
| 4 | 800 | 12566.3 | 0.62 |
| 5 | 1000 | 15623.9 | 0.62 |

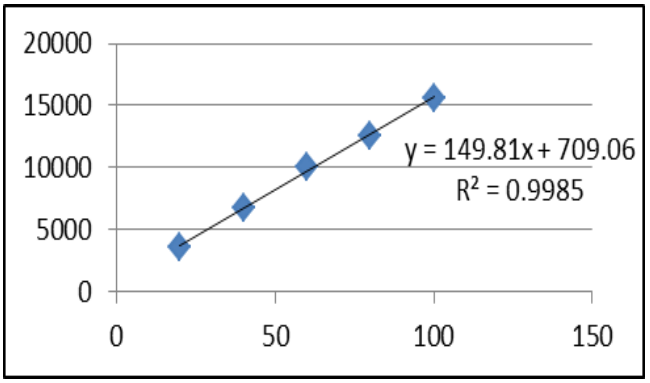


**Fig. 4: Linearity graph of cetilistat.**

**LOQ AND LOD**

LOD and LOQ were calculated from the equation and were found to be 0.9094ng/spot and 2.7558 ng/spot respectively.

**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 100.10% to 101.33% and was shown in Table 2.

**Table 2: Recovery studies n=3.**

| Sr no | Level of % recovery | Amount spiked (ng/band) | Average peak area | Amount found (ng/band) | % Recovery | Mean % recovery | %RSD |
|---|---|---|---|---|---|---|---|
| 1 | 80 | 108 | 17104.7 | 109.44 | 101.33% | 100.74% | 0.4669 |
| 2 | 100 | 120 | 18704.7 | 120.12 | 100.10% | | 1.7505 |
| 3 | 120 | 132 | 20642.5 | 133.05 | 100.80% | | 1.2982 |

**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 3 and 4.

**Table 3: Interday precision n=3.**

| Sr. no. | Concentration (ng/band) | mean area | Standard Deviation | %RSD |
|---|---|---|---|---|
| 1 | 400 | 6655.6 | 115.798 | 1.739858 |
| 2 | 600 | 10041.7 | 73.157 | 0.728534 |
| 3 | 800 | 12385.47 | 223.494 | 1.804491 |

**Table 4: Intraday precision n=3.**

| Sr no | Concentration ( ng/band) | mean area | Standard Deviation | %RSD |
|---|---|---|---|---|
| 1 | 400 | 6673.5 | 41.284 | 0.618637 |
| 2 | 600 | 10045.57 | 49.521 | 0.492973 |
| 3 | 800 | 12322.63 | 215.683 | 1.750301 |

**Assay**

The drug content in the marketed formulation was found to be 100.26%. There was no interference of excipients in the marketed formulation. Table 5 indicates results obtained in the determination of cetilistat in pharmaceutical dosage form.

**Table 5: Assay of pharmaceutical dosage form.**

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

**Robustness**

Robustness was carried out by deliberate modification of analytical parameters, which indicated that $R_f$ value and peak area remained unaffected by small changes in wavelength, mobile phase volume and chamber saturation time. %RSD calculated was with in ICH limit thus indicating that method was sufficiently robust. The results were shown in Table 6.

**Table 6: Robustness study of cetilistat.**

| Parameter | Change in parameter | % Estimation | Mean | S.D | %RSD |
|---|---|---|---|---|---|
| Wavelength (± 2nm) | 226 | 100.52 | 100.67 | 0.513 | 0.509 |
| | 228 | 100.26 | | | |
| | 230 | 101.25 | | | |
| Mobile phase volume (± 2%) | 12ml | 100.58 | 100.16 | 0.472 | 0.471 |
| | 10ml | 100.26 | | | |
| | 8ml | 99.65 | | | |
| Saturation time (± 2 minutes) | 22 min | 101.54 | 100.82 | 0.653 | 0.648 |
| | 20 min | 100.26 | | | |
| | 24 min | 100.67 | | | |

## CONCLUSION

The proposed HPTLC 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, and reproducible. The proposed method was 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 bulk and pharmaceutical dosage form.

## ACKNOWLEDGEMENT

The authors are thankful to the Management and Principal of Allana College of Pharmacy for encouragement to carry out the work as well as for providing the facilities to carry out the research work.

## REFERENCES

1. Kang JG, Park CY. Anti-obesity drugs: a review about their effects and safety. Diabetes & metabolism journal, 2012; 36(1): 13-25.
2. Baheerati M, Devi G. Obesity in relation to Infertility. Research J. Pharm. and Tech, 2018; 11(7): 3183-3185.
3. Kopelman P, Bryson A, Valensi P. Cetilistat (ATL-962), a novel lipase inhibitor: a 12-week randomized, placebo-controlled study of weight reduction in obese patients. International Journal of Obesity, 2006; 31(3): 494-499.
4. Yamada Y, Kato T, Ogino H, Shuntaro A. Cetilistat (ATL-962), a Novel Pancreatic Lipase Inhibitor, Ameliorates Body weight Gain and Improved Lipid Profiles in Rats. Hormone and Meatabolic Research, 2008; 40(8): 539-43.
5. J Gras. Cetilistat for the treatment of obesity. Drugs Today (Barc), 2013; 49(12): 755-9.
6. Padwal R Cetilistat, a new lipase inhibitor for the treatment of obesity. Curr Opin Investig Drugs, 2008; 9(4): 414-21.
7. Kshirsagar S.A, Mane S.B, Hanchate Y.S, A. Katte S. UV Spectrophotometric Method Development and Validation for Determination of Cetilistat in API and in Pharmaceutical Dosage Form. International Journal for Pharmaceutical Research Scholars, 2018; 7(1): 1-8.
8. Jadhav S, Morey P, Karpe M, Kadam V. RP-HPLC Method Development and Validation for Determination of Atenolol in Bulk Drug. Research J. Pharm. and Tech, 2013; 6(7): 736-739.
9. Daharwal S.J, Shrivastava S. HPTLC Method Development and Validation for Quantification of Quercetin in Thuja occidentalis Mother tincture. Research J. Pharm. and Tech, 2019; 12(9): 4102-4106. doi: 10.5958/0974-360X.2019.00707.8
10. Tamilselvi N, Arivukkarasu N, Sasikala R, Shahanas, Shamina, Sreelakshmi J. Development and Validation of HPTLC method for the Determination of Efavirenz in Tablet Dosage Form. Research J. Pharm. and Tech. 2018; 11(3): 885-888.
11. Bharskar G, Mankar S, Siddheshwar S. Analytical Methods for Estimation of Curcumin in Bulk, Pharmaceutical Formulation and in Biological Samples. Asian Journal of Pharmaceutical Analysis, 2022; 12(2): 142-8. doi: 10.52711/2231-5675.2022.00025.
12. Edlabadkar A.P, Rajput A.P. RP-HPTLC Method for Determination of Garenoxacin mesylate in Bulk and in Tablet Formulation. Asian J. Pharm. Ana, 2018; 8(2): 78-82. doi:10.5958/2231-5675.2018.00015.7
13. Rajasekaran A, Arivukkarasu R, Linda M. Estimation of Andrographolide Content in Aqueous Extract of Siddha Formulations by HPTLC. Asian J. Pharm. Ana, 2015; 5(4): 206-208.
14. Ramasamy A, Aiyalu R. Fingerprint Analysis of Herbal Raw Materials available in Market Belongs to Combretacea And Fabaceae family by HPTLC technique using antioxidant markers. Asian Journal of Pharmacy and Technology, 2021; 11(2): 130-4.
15. Bhaskar O.A, Yogesh T.S, Vinod A.B, Parag A.P. HPTLC Method for Estimation of Ellagic Acid and Gallic Acid from Flowers of Woodfordia fruticosa Kurz. Asian Journal of Research in Pharmaceutical Sciences, 2021; 11(3): 187-2. doi: 10.52711/2231-5659.2021.00029
16. Vidhya K.B, Sunil R.D. Development and Validation of a Stability-Indicating HPTLC Method for the Estimation of Eszopiclone in Pharmaceutical Dosage Forms. Asian Journal of Research in Pharmaceutical Sciences. 2021; 11(3): 219-3.
17. Salunke C, Chittam K, Pawar A, Chaudhari H. Development of an Analytical Method to Determine Extracts of Different Species of Glycyrrhizin by HPTLC. Res. J. Pharma. Dosage Forms and Tech, 2020; 12(2): 89-92.
18. Chaudhry S, Patil B.R. Stability Indicating Analytical Method Development and Validation for Estimation of Orlistat in Bulk and its Dosage form by HPTLC Technique and Finding Degradants by LC-MS. Am. J. PharmTech Res, 2018; 8(3): 1-15.
19. Gurdeep R.C and Sham K.A. Instrumental Method of chemical Analysis. HPTLC, 2. 599-2. 616, HPLC: 2.624-2.639.
20. ICH, Q2 (R1) Validation of Analytical Procedures: Text and Methodology. ICH Harmonized Tripartite Guideline, 2005; 8–13.