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Research Article

DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING METHOD FOR ESTIMATION OF CETILISTAT IN BULK AND TABLET MARKETED FORMULATION BY USING RP-HPLC AND SPECTROSCOPY METHOD IN FORCED DEGRADATION EXPERIMENTS.

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ABSTRACT:
In the current study, estimation of cetilistat by Uv spectroscopy, reverse -phase Chromotography method in pharmaceutical dosage form and bulk form was developed, In the Uv-Visible Spectroscopy method cetilistat was quantified at 306 nm absorption maxima using 6.5 ph Phosphate Buffer as the solvent. In the RP-HPLC method, the chromatogram was run through a SunFire C18 Column (100Å, 5.0 µm, 4.5 mm X 150 mm) with a mobile phase of 0.01N potassium dihydrogen phosphate: methanol in a 70:30 ratio. The temperature was kept at 30°C and the optimized wavelength was 306.0nm. The flow rate was 0.6ml/min, Analytical calibration curves by Uv were linear within a concentration range from 7.5 to 22.5 µg/ml and coefficient of correlation 0.999, Retention time of Cetilistat was found to be 2.483 min. %RSD of the Cetilistat were and found to be 0.5%. %RSD of Method precision of Cetilistat was found to be 0.9%. %Recovery was obtained as 100.26% for Cetilistat. LOD, LOQ values obtained from regression equation of Cetilistat were 0.22, 0.68, Regression equation of Cetilistat is y = 39410x + 46549, The procedures were validated using the International Conference on Harmonization's (ICH) standard Q2 (R1). The degradation conditions were used in accordance with ICH recommendations Q1A(R2) and Q1B, which include acid, alkaline, neutral, thermal, and photostability, to test the drug's intensity of stability, Based on analytical results, the HPLC method is best for cetilistat quantification tablet formulation due to its high reproducibility, good retention time and sensitivity; it has a higher percent recovery and has less analysis time, i.e., 5 min. The degradation peaks were well separated from the cetilistat peak indicating stability of the HPLC method.

Key Words: Cetilistat, RP-HPLC, Uv-Visible Spectroscopy, Method Development, Validation, Forced Degradation.

INTRODUCTION

Obesity and overweight provide major health risks to both individuals and communities, Obesity produces not only visual problems, but also improper physiological metabolism, which leads to a variety of physiological, psychological, and social issues.[1,2] Obesity is a significant risk factor for a variety of diseases, including cardiovascular disease, hypertension, hyperlipemia, diabetes, and even cancer, and it is linked to the formation of many chronic diseases. Obese people are more likely to have type 2 diabetes, gallbladder disease, and syndrome than people of normal weight. Coronary heart disease, hypertension, osteoarthritis, and gout all raise the risk of obesity, and there are some reproductive implications as well, Pancreatic lipase inhibitors play an important role in the metabolism of human fat.[3] It converts the oil in the diet into small molecules of glycerol and fatty acids, which the body may absorb and participate in metabolism.[4,5]

Cetilistat chemically Fig-1 known as (2-hexadecoxy-6-methyl-3,1-benzoxazin-4-one), It possesses a high concentration of Benzoxazine derivative, which acts as an inhibitor of gastric and pancreatic lipase.[6] Pancreatic lipase, one of the key enzymes involved in dietary fat breakdown, works predominantly on the duodenum and proximal jejunum, inhibition of pancreatic lipase reduces weight by decreasing dietary fat absorption, therefore limiting calorie intake to the body, this enzyme is essential for the digestion of dietary lipids, breaking down

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long-chain triglycerides into absorbable free fatty acids and monoglycerides.[7] Cetilistat was granted FDA approval on 2018.[8]

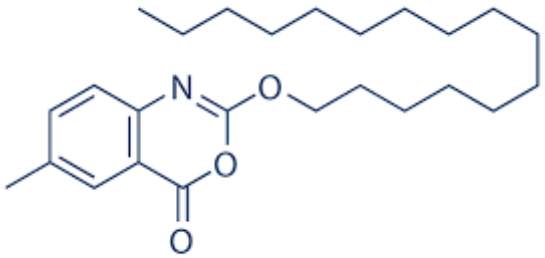

Fig 1: Chemical Structure of Cetilistat

An exhaustive literature study revealed that Cetilistat has been determined separately by UV spectroscopy and RP-HPLC[8,9,10,11]. However, no single study has reported that Cetilistat was found to be stable, indicating that a study of the selected superior method for separating the active analyte present in the pharmaceutical dosage is being conducted, making the current study unique and novel. As a result, we have worked hard to develop precise, accurate, sensitive, and cost-effective procedures and compare them based on analytical outcomes such as sensitivity, percentage recovery, and drug assay. The approach was validated in accordance with ICH Q2 (R1) requirements.[12]

MATERIALS AND METHODS

Chemicals and reagents

Cetilistat standard (Purity ≥ 99.8 as is basis), Acetonitrile (HPLC grade), HPLC grade water (Millipore), 0.1% Orthophosphoric acid, Potassium hydroxide, Hydrochloric acid and Hydrogen peroxide were purchased from merck.

Instrumentation: The instrument used in the study were electronic balance (Make: Mettler Toledo, Model: XP56), sonicator (Make: Elma, Model: S300H), hot air oven (Make: Serve well Instruments, Model: H02436), digital meter (Make: Mettler Toledo) and UV-Visible chamber (Make: Mack Equipment, Model: MK-2). HPLC (Waters 2695 with PDA detector 2996) was monitored and integrated using Empower 3 software, A double-beam UV-1800 Shimadzu UV spectrophotometer along with a pair of matched quartz cells 10 mm is used

Sample preparation for UV and RP-HPLC method development

Diluent: Based up on the solubility of the drugs, diluent was selected, first dissolved in Methanol and water taken in the ratio of 50:50.

Buffer preparation:

Buffer: (0.1N potassium dihydrogen orthophosphate)

Dissolve 6.8 g of potassium dihydrogen orthophosphate in 1000 mL of water and adjust the pH to 6.8 with 1M sodium hydroxide (solution A).

Preparation of Standard stock solution:

Accurately weighed 15mg of Cetilistat was transferred to 100ml volumetric flask. 50ml of diluent was added to the flask and sonicated for 10mins. Flask was made up to the mark with diluent and labelled as standard stock solution. (150µg/ml of Cetilistat).

Preparation of Standard working solution: 1ml from stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent (15µg/ml of Cetilistat)

Preparation of Sample stock solution:

10 Kilfat tablets (label clam per one tablet: 60 mg of Cetilistat) was weighed and average weight of each tablet was calculated. Then weight equivalent to 1 tablet was transferred into a 200 ml volumetric flask, 100ml of diluent added and sonicated for 15 min, further the volume was made up to the mark with diluent and filtered with PVDF nylon filters. (300µg/ml of Cetilistat).

Preparation of Sample working solution: From the filtered solution, 0.5ml was pipetted out into a 10 ml volumetric flask and made up to 10ml with diluent. (15µg/ml of Cetilistat).

Determination of Cetilistat maximum absorbance (λmax)

A typical cetilistat working solution in the range of 400-200 nm is scanned. The absorption maxima was found to be 306 nm, which was chosen as the analytical wavelength for future investigation. The spectrum was recorded as indicated in Figure 2.

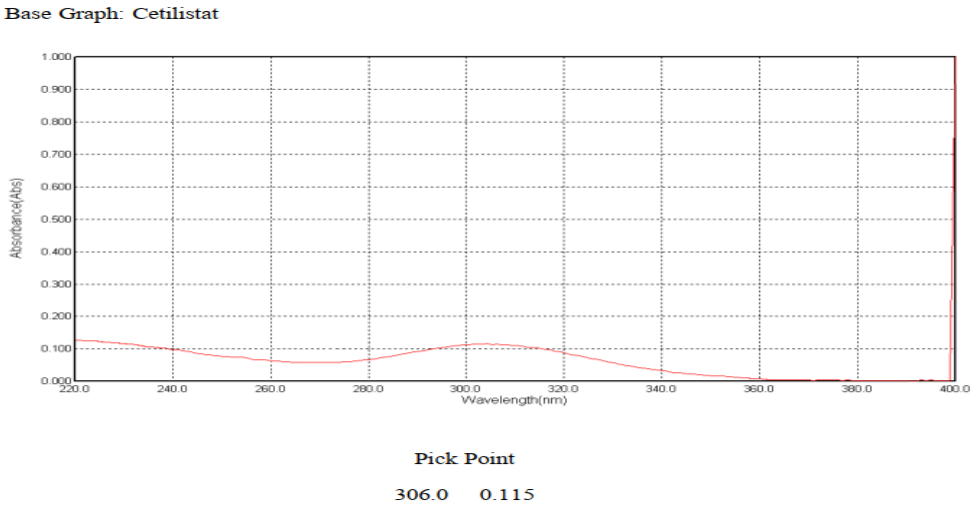

Fig 2. Spectra of Cetilistat

HPLC method development

To achieve optimized chromatographic conditions, the below parameters were modified in each trial. The trial runs are shown in Table 1.

Table: 1 Trial run for optimization of Cetilistat

Trails	**Mobile phase, Stationary phase**	**Flow rate (mL/min)**	**Retention time (min)**	**Comment**
Trail-1	Kromosil 150 (150mm x4.5 mm, 3.7µ). ACN : Kh2Po4(60:40v/v)	1.0 ml/min	6.606 min	peak shape was not good and baseline disturbance
Trail-2	BDS 150 (150mm x4.5 mm, 5m). ACN: Kh2Po4 (50:50v/v)	1.0 ml/min	2.747 min	peak splitting and broad peak shape was observed
Trail-3	BDS 150 (150mm x4.5 mm, 5m). Methanol : Water(60:40v/v)	0.5 ml/min	7.212 min	broad peak shape and peak asymmetry is observed
Trail-4	Sunfire150 (150mm x4.5 mm, 5m). Methanol: Kh2Po4 (80:20v/v)	0.4 ml/min	3.148 min	all the suitability parameters were passed

From the trials, the best possible optimized chromatographic condition was selected based on peak shape that is sharp evaluated by theoretical plates and tailing factor which were within specified limit, and retention time is 2.483 min which is much less. Therefore, separation of Cetilistat was performed on a Sunfire150 (150mm x4.5 mm, 5m). consisting of Methanol: Kh2Po4 (30:70v/v) as a mobile phase; by using a membrane filter it was filtered and degassed. The flow rate was retained at 0.6 mL/min. The injection volume was kept at 10 µl at a column oven temperature of 30 °C, and analyte eluted at detected at 271 nm. The mode of separation was isocratic. The chromatogram of Cetilistat is shown in Fig no 3 respectively.

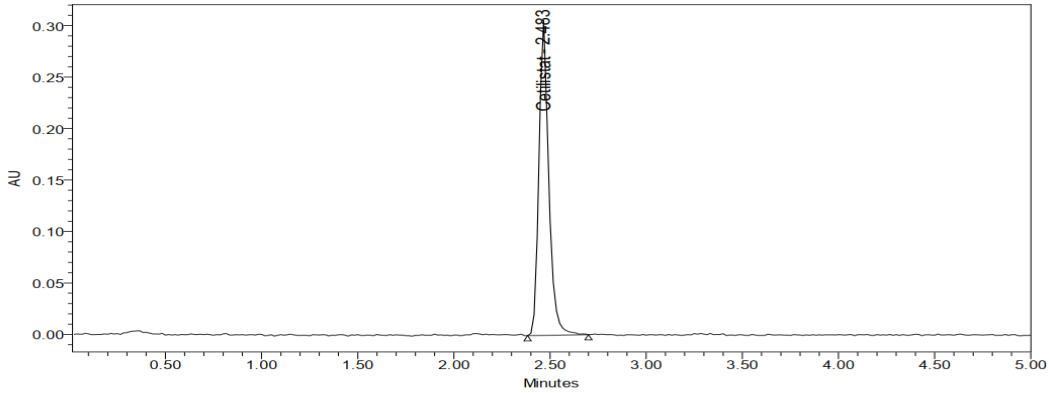

Fig 3 Optimized Chromatogram

Method Validation: The method was validated in accordance with ICH recommendations Q2R1. System appropriateness, specificity, linearity, accuracy, precision, LOD& LOQ, and robustness are among the validation parameters.

RESULTS AND DISCUSSION

System suitability parameters: The system suitability parameters were assessed by making standard solutions of Cetilistat (10µg/ml) and injecting them six times. Peak tailing, resolution, and USP plate count were all determined. For three medications in combination, the USP Plate count exceeded 2000 and the tailing factor was less than 2. All the system's appropriate parameters were passed and remained within the limitations. Table 2 shows the results.

Table: 2 System suitability parameters for Cetilistat

S no	Cetilistat		
Inj	RT(min)	USP Plate Count	Tailing
1	2.439	9326	1.53
2	2.440	9373	1.54
3	2.441	9327	1.55
4	2.442	9374	1.55
5	2.452	9395	1.52
6	2.452	9328	1.52

Specificity: In the optimised method, the interference is checked. Cetilistat, had retention time of 2.483 minutes. We did not found any interfering peaks in the chromatograms of blank and placebo samples during the retention periods of the drug in our approach. As a result, this procedure was stated to be. Figures 4 show the chromatograms for specificity.

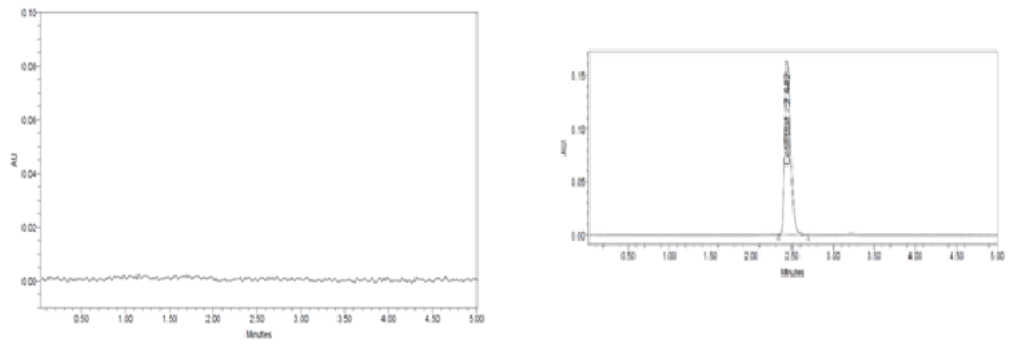

Figure No.4 Specificity Chromatograms

Linearity: Six linear concentrations of Cetilistat (3.75-22.5µg/ml) was injected in duplicate manner. Correlation coefficients obtained was 0.999 for all the three drugs. The results were shown in table 3 and fig 5.

Table 3 Linearity table for Cetilistat.

Cetilistat	
Conc (µg/mL)	**Peak area**
0	0
3.75	196905
7.5	346566
11.25	485615
15	627411
18.75	788291
22.5	938011

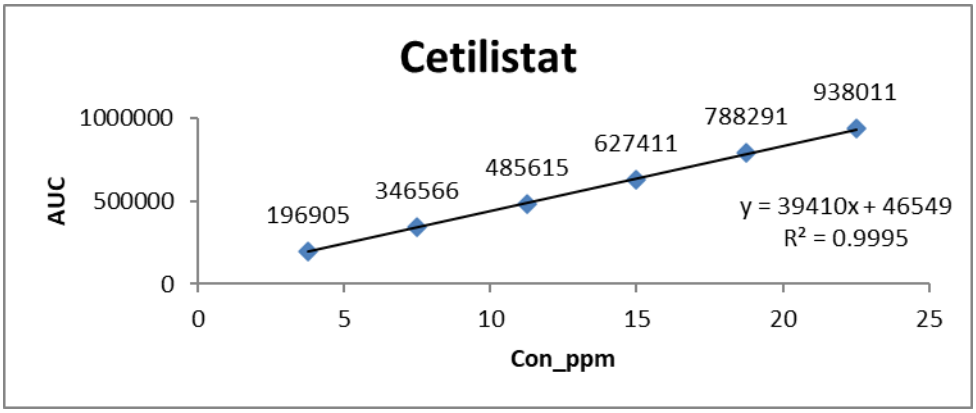

Fig No.5 Calibration curve of Cetilistat

Precision:

Repeatability: Multiple samples were taken from a sample stock solution, and six working sample solutions of the same concentrations (15µg/ml Cetilistat) were created. Each injection was given from each working sample solution, and the results are shown in table 3. The average area, standard deviation, and % RSD for the medication were computed and found to be 0.9% for Cetilistat. The system precision was passed for this procedure since the precision limit was less than "2 %." Table 4 shows the information results.

Table 4 Repetability table of Cetilistat

S. No	Area of Cetilistat
1.	624556
2.	623567
3.	629665
4.	619565
5.	620655
6.	613557
Mean	**621928**
S.D	**5417.2**
%RSD	**0.9**

Intermediate Precision: Multiple samples were taken from a sample stock solution, and six working sample solutions of the same concentrations (15µg/ml of Cetilistat) was prepared. Each injection from each working sample solution was given on the following day of the sample preparation, and the obtained areas are listed in table 4. The average area, standard deviation, and % RSD for the medication was computed and found to be

0.3% for Cetilistat. Because the precision limit was less than "2%" the intermediate precision was used for this procedure. Table 5 shows the information results.

Table 5 Intra-Day precision table of Cetilistat

S. No	Area of Cetilistat
1.	625656
2.	624567
3.	624767
4.	625867
5.	630767
6.	625678
Mean	626217
S.D	2091.9
%RSD	0.3

Accuracy: The conventional addition procedure was used to create three levels of accuracy samples. Triplicate injections were administered at each degree of accuracy, and the mean % recovery for Cetilistat was found to be 100.26 %. Tables 6 show the outcomes. Because satisfactory recover values were achieved, the accuracy for this approach was passed.

Table 6 Accuracy data of Cetilistat

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	7.5	7.60	101.38	100.26%
	7.5	7.36	98.10	
	7.5	7.36	98.13	
100%	15	15.14	100.94	
	15	15.26	101.74	
	15	15.12	100.80	
150%	22.5	22.50	100.02	
	22.5	22.51	100.05	
	22.5	22.76	101.16	

Robustness: Robustness conditions such as flow minus (0.9ml/min), flow plus (1.1ml/min), mobile phase minus (65:35 v/v), mobile phase plus (55:45 v/v), temperature minus (25°C), and temperature plus (35°C) were maintained, and samples (50µg/ml Cetilistat) was injected in triplicate. The % RSD was computed and determined to be within the acceptable range. Table 7 shows the data.

Table 7 Robustness data of Cetilistat

S.no	Condition	%RSD of Cetilistat
1	Flow rate (-) 0.5ml/min	0.3
2	Flow rate (+) 0.7ml/min	0.2
3	Mobile phase (-) 65B:35A	0.3
4	Mobile phase (+) 75B:25A	0.2
5	Temperature (-) 25°C	0.5
6	Temperature (+) 35°C	0.4

Assay: Kilfat bearing the label claim Cetilistat 200mg. Assay was performed with the above formulation. Average % Assay for Cetilistat obtained was 99.45%. The chromatogram is depicted in Fig. 6.

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{1}{10} \times \frac{200}{1} \times \frac{10}{0.5} \times \frac{p}{100} \times \frac{AvWt}{L.C} \times 100$$

AT	Average Peak area of in test solution
AS	Mean peak area of in standard solution
WS	Weight of working standard taken in mg
P	Potency of working standard in % on dried basis
L.C	Label Claim
Avwt	Average weight of drug

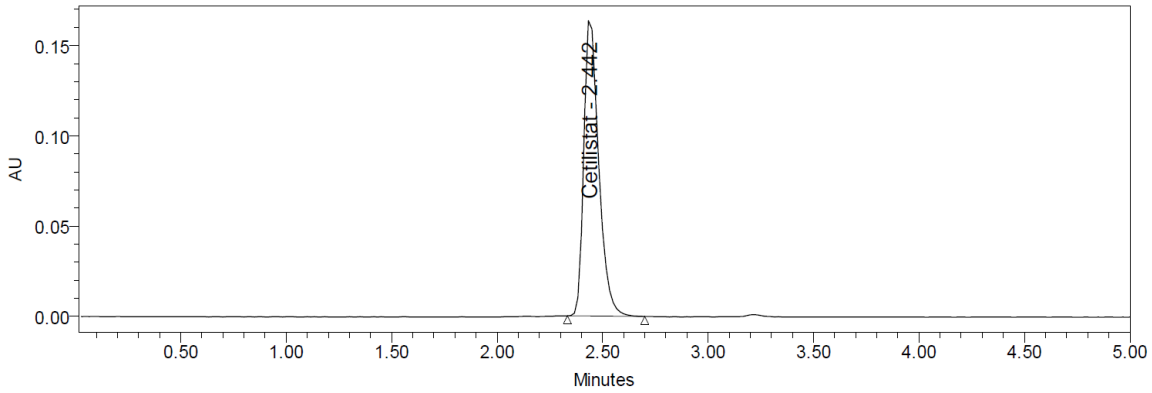

Fig No.6:Sample Chromatogram

Degradation: To conduct the forced degradation experiment, standard stock solutions of Cetilistat was exposed to various stress conditions, including 1 mL of 20% H2O2 (for oxidative degradation), 1 mL of 2N HCL (for acidic degradation), and 1 mL of 2N NAOH (for acidic degradation) (for basic degradation). The produced solutions were refluxed for 30 minutes at 60oC. To examine the descent, the standard solutions were also subjected to UV radiation and temperature conditions.13 The resulting solutions were diluted to yield 15µg/ml of Cetilistat for degradation studies. To examine sample stability, 10µl samples were fed into the system and chromatograms were obtained, there is no interaction of the degradation peak with that of the cetilistat peak. Hence, the proposed HPLC method was stability indicating and specific. The peak purity index values of cetilistat peak and degradation peaks were found to be within acceptable limits, Table 8 and fig no 7, 8.

Table No.8: Degradation Data

S.No.	Condition	Cetilistat %Degraded	Cetilistat %Obtained	Purity angle	Purity Threshold
1	Acid	4.72	95.28	0.261	0.447
2	Base	6.91	93.09	0.204	0.373
3	Oxidation	1.53	98.47	0.205	0.354
4	Dry heat	1.69	98.31	0.213	0.331
5	UV Light	0.85	99.15	0.160	0.357
6	Hydrolytic	0.41	99.59	0.165	0.352

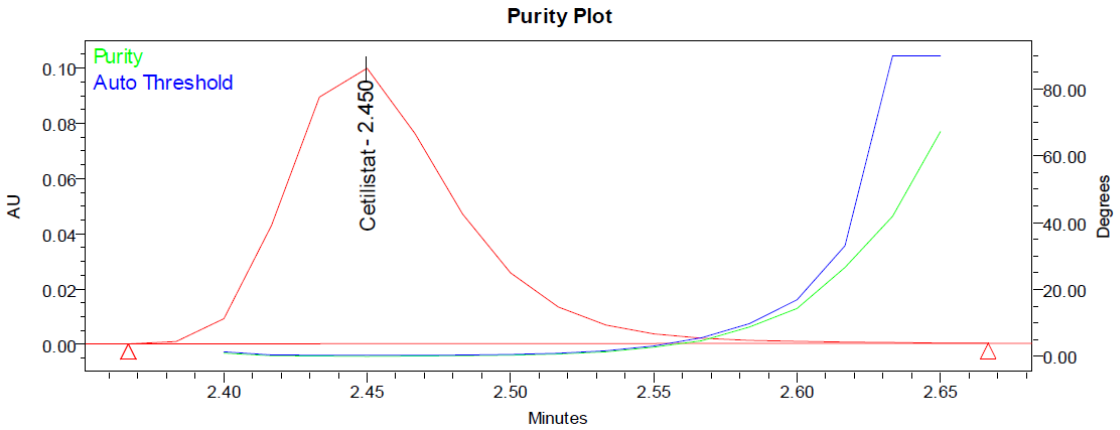

Fig No 7: Acid Purity plot

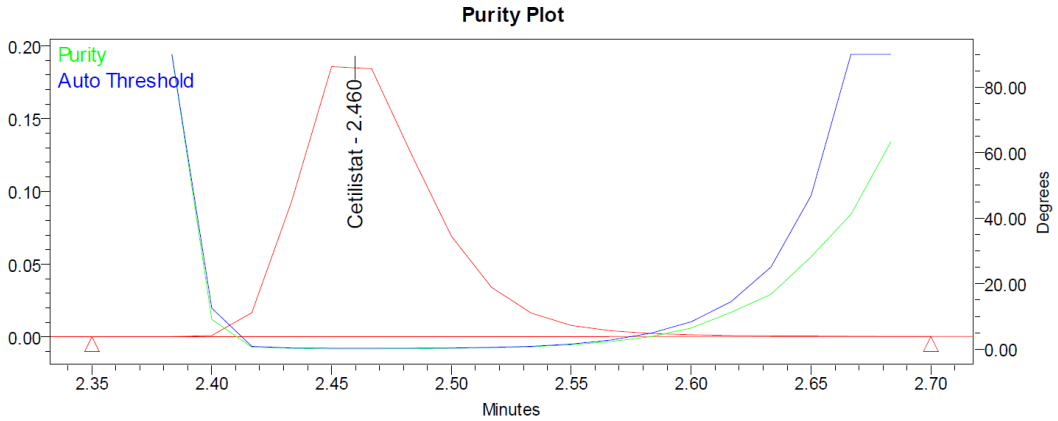

Fig No 8: Base Purity plot

CONCLUSION:

This work shows how to determine Cetilistat in the presence of substances using a simple and established stability-indicating RP-HPLC approach. The devised approach was exact, sensitive quick, and durable. The approach is capable of distinguishing active medicinal components from degradation products generated during forced degradation tests. The suggested approach may be utilized for routine quantitative Cetilistat analysis in the quality-control department.

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