Title Name:- UV AND RP-HPLC METHOD DEVELOPMENT OF CETILISTAT IN TABLET DOSAGE FORM AND IT'S VALIDATION

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ABSTRACT

Developing and validating a UV and RP-HPLC technique to analyze Cetilistat tablets is the primary objective of this project. In order to use the UV spectrophotometric approach, Cetilistat solutions were produced using n-hexane. Quartz cuvettes measuring 1 cm were used to scan the Cetilistat standard solution in the 400-200 nm UV spectrum.

The RP-HPLC method, which employs a ziodiac C18 column (150 mm*4.6 mm*3 µm) and a solvent combination of 50:50 acetonitrile and water, was used to identify the anti-obesity drug. A 0.45 µm membrane filter was used to filter the mobile phase after a brief sonication-based degassing process. When doing drug analysis, one of the mobile phases used is a 50:50 combination of acetonitrile and phosphate buffer with a pH of 4.4. The antiobesity medication was detected at 210 nm using a Photodiode Array Detector at 30 °C and 1.0 mL/min. It was separated in less than five minutes.

Key Words: Cetilistat, Antiobesity, Validation, RP-HPLC, Isocratic

INTRODUCTION

A study published in the Lancet highlights concerning obesity rates in India, indicating that 70% of the urban population is classified as overweight. India ranks third globally in obesity, following the United States and China. Approximately 80 million persons in India are considered obese, with around 10 million of them aged between 5 and 19 years.[1-2]

Synthetic medications used for the treatment of obesity include Sibutramine, Orlistat, Phentermine, Diethylpropion, Fenfluramine, and Liraglutide.[3]

1.1 OUTLINE TO ANALYTICAL CHEMISTRY:

Analytical chemistry investigates and employs equipment and procedures that are experienced in material separation, classification, and calculation. The ideal analysis can be arranged using identification, separation, preparation, and evaluation alone or in combination with another method.[4]

UV- visible Spectroscopy:

One analytical method is UV-Visible spectroscopy, which analyzes the absorption or transmission of certain wavelengths of ultraviolet (UV) or visible (visible) light by a material. With respect to a control or reference sample.[5-6]

Beers-Lambert Law:

According to the Beer-Lambert equation, the light absorbance is exactly proportional to the sample's concentration and route length for a particular material.[7]

CHROMATOGRAPHY:

Chromatography is a continuous two-phase separation technique for chemical mixtures. There is a constant transition between the mobile and stationary phases.

Principle of Chromatography:

- **Partition Chromatography**
- **Adsorption Chromatography [8]**

HPLC

One method for separating substances from a combination is HPLC. There are two parts to the method: the stationary phase and the mobile phase.Different distribution coefficients for the two phases allow for component separation.

Principle of HPLC:

The following types of HPLC are distinguished by the substrate, or stationary phase, that is used:

1) **Normal Phase HPLC**: Hexane, chloroform, and diethyl ether are examples of non-polar phases, while polar silica is often utilized as the stationary phase. The column keeps the polar samples.
2) **Reverse Phase HPLC**: More its non-polarity, the greater its retention.
3) **Size-exclusion HPLC**: Substrates will be added to the column in a regulated fashion.
4) **Ion-exchange HPLC**: the sample charge is opposed to an ionically charged surface on the stationary phase. To regulate the pH and ionic strength, an aqueous buffer is employed as the mobile phase. [9]

Methodology: Validation of Parameter:[10]

1. Specificity
2. LOD and LOQ
3. Linearity
4. Accuracy
5. Precision
6. Range
7. Robusrness
8. Ruggedess
9. Repeatability

MATERIALS AND INSTRUMENTS

A. Drug acquirement

Name of drugs	**Drug Obtained**
Cetilistat API	Dhamtec Pharma and Consultant Navi Mumbai.

Table No.1: Drug Acquirement

B. Reagents and synthetic Substances Used

Chemicals / Reagents	Make	Grade
Acetonitrile	Merk or Equivalent	HPLC
Water	-	HPLC
Orthophosphoric Acid	Merck or Equivalent	AR grade
Sodium Dihydrogen Phosphate monohydrate	Merck or Equivalent	AR grade

Table No. 2: Reagents and Synthetic Utilized

C. Instruments Utilized

Parameter	Details
Chromatographic System	Isocratic System
Pump	Reciprocating Pump – Thermo Scientific
Detector	DAD 3000
Software	Chromeleon 7
Column	Zodiac C18, 150 mm × 4.6 mm × 3 µm
Analytical Balance	Mettler Toledo – Digital Analytical Balance
pH Meter	Horiba – Digital pH Meter
Sonicator	Ultrasonic Bath Sonicator
Filter	Nylon/PTFE 0.45 µm (Unichrome or Equivalent)

Table No. 3: Instruments Utilized

EXPERIMENTAL WORK

UV Spectrophotometric Method:

Selection of Solvent System:

A variety of solvents, including water, methanol, 0.1N HCL, 0.1 N NaOH, and others, were tested for the drug's solubility before being chosen. The medicine dissolved easily in n-Hexane.We chose n-Hexane as our solvent solution for UV methid development because of its solubility and because it was inexpensive.

Preparation of Standard Stock Solution

The drug's standard stock solution was prepared using a volumetric flask that contained 5 milliliters of n-hexane and 10 milligrams of accurately weighed Cetilistat. Ensure the medicine is mixed properly. I used n-hexane to get the volume to the desired level, and then I produced a concentration of 1000 µg/ml. The standard stock solution, consisting of 100 micrograms per milliliter, was prepared by transferring 2 milliliters of the solution to a 20 milliliter volumetric flask and filling it up with n-hexane. Concentrations between twenty and one hundred micrograms per milliliter are achieved by further diluting it with n-hexane.

Selection of Wavelength:

The drugs was soluble in n-Hexane, Prepared different concentrations of solution. These solution scan or recorded spectrum between 200-400 nm using n-Hexane as a blank. In UV Spectrophotometric method, wavelength 210 nm was certain for determination of Cetilistat

Calibration Curve for Cetilistat (2.5–12.5 µg/ml):

A number of 10 ml volumetric flasks were precisely filled with an appropriate volume of the standard Cetilistat stock solution. The final concentrations were 2.5, 5, 7.5, 10, and 12.5 µg/ml, which were achieved by adjusting the volumes with water to the mark. By graphing absorbance versus concentration, a calibration curve was created using absorbance values taken at 239 nm. The calibration curve was represented by a straight-line equation that was calculated.

Method Validation:

Method validation is conducted to authorize that analytical procedure developed demonstrates suitable performance characteristics for its intended analytical application.

Linearity:

Linearity was evaluated by preparing a series of standard solutions of Cetilistat ranging from 2.5 to 12.5 µg/ml. A calibration plot of absorbance against concentration was generated for these solutions.

Precision:

Studies were conducted both within and between days to see how accurate the new procedure was. Three analyses of the standard working solution were performed in a single day to determine the percentage relative standard deviation (% RSD), which allowed for intraday accuracy. We recalculated the percentage RSD after analyzing the identical solution for two days in a row to ensure interday accuracy.

Accuracy:

Ten tablets were weighed and finely crushed into powder. After careful weighing, 100 milligrams of Cetilistat was added to a 100 milliliter volumetric flask. To proceed, 50 ml of n-Hexane was added and the mixture was sonicated for 15 minutes. After shaking, n-Hexane was added until the volume was filled to the mark. After that, the solution was filtered using Whatman filter paper (0.45 µm). A 10 milliliter volumetric flask was used to create a solution with a concentration of 100 µg/ml by transferring 1 milliliter from the finished solution. The next step was to add water to dilute it. Again, 1 mL of this solution was transferred to a separate 10-mL flask and diluted to make a 10-microgram/mL solution of Cetilistat. . Absorbance was measured at 210 nm. Accuracy was assessed at 80%, 100%, and 120% levels using both the standard and marketed formulation. The percentage recovery was calculated and found acceptable per ICH guidelines.

LOD and LOQ:

According to ICH guidelines, various methods exist to determine detection and quantification limits. In this case, the third approach was used, with the formulas applied as follows:

Robustness:

Robustness was assessed by analyzing standard solutions under slightly varied but intentional modifications to the analytical parameters. The wavelength variation was selected as the test factor for this evaluation.

Ruggedness:

Ruggedness was verified by having different analysts perform the procedure. The method produced consistent results regardless of the analyst performing the test.

RP-HPLC Method:

Instruments:

method development and validation were conducted using an Agilent HPLC system that included a quaternary pump, sample injector with a 10 µl loop, PDA detector, and Zodiac C18 column (250 mm × 4.6 mm, 5 µm). Chromeleon 7.2 software was utilized for data collection and peak quantification.

Optimized chromatographic condition:

Preparation of 2%v/v Orthophosphoric Acid:

Transfer 2 mL conc. Orthophosphoric Acid to 100 mL with water and mix.

Preparation of phosphate buffer pH 4.4:
Weigh and dissolve 1.36 g of sodium dihydrogen phosphate monohydrate to 1000 ml water, and adjust pH 4.4 with 2%v/v Orthophosphoric Acid. Filter through 0.45 µm Nylon member filter paper.

Mobile Phase:
Mix phosphate buffer pH 4.4 and acetonitrile in the ratio of 50:50 % v/v

Diluent:
Mix water and acetonitrile in the ratio 50:50 % v/v

Standard Preparation (conc. 20 ppm):
Weigh and transfer accurately about 25 mg of Cetilistat standard in to 250 ml volumetric flask. Add about 150 ml of diluent, sonicated to dissolve. Make up to mark with same diluent and mix well. Dilute 5 ml of above solution to 25 ml with same diluent and mix well.

Assay preparation (conc. 20 ppm):
Weigh 20 tablets and calculate average weight. Weigh and crush tablets and transfer powder equivalent of about 100 mg Cetilistat into 200 ml of volumetric flask. Add 150 ml of diluent, Centrifuge the sample for 10 min at 5000 rpm then filter the solution through 0.45 µm PVDF filter. Dilute 4ml of filtrate to 100 ml with diluent..

Procedure:

Inject 10 µL of blank, standard (5 replicates), and sample. Evaluate peak responses for Cetilistat.

System suitability: %RSD ≤ 2.0, tailing factor ≤ 2.0.

Method Validation (as per ICH Q2B)

Linearity:

Prepare 6 levels (25–150% of 20 ppm). Create stock solution (199.92 ppm) and dilute to desired concentrations.

Precision:

Inject 5 replicates of standard for system precision. Method precision assesses reproducibility using multiple sample preparations.

Accuracy:

Assess at 50%, 100%, 150% by spiking placebo with Cetilistat and analyzing recovery.

Specificity:

Ensure no interference at Cetilistat RT from blank or placebo.

Robustness:

Evaluate method stability with slight variations in parameters.

Ruggedness:

Assess results consistency with different analysts.

Repeatability:

Evaluate intra-day precision under same conditions.

Solution Stability:

Compare standard (up to 24 h) and sample (up to 33 h) at 25°C. Monitor assay % change and similarity factor over time.

RESULTS AND DISCUSSION

Method Validation of UV Method Development:

Linearity:

Calibration curve for Cetilistat:

Parameters	Observation
λmax,Wavelength(nm)	217 nm
Linearity range(µg/ml)	2.5-12.5

Slope	0.0801
Intercept(c)	0.0017
Regression coefficient(R^2)	0.9998

Table No.3 Optical characteristics and other parameter

Concentration(µg/ml)	Absorbance(217nm)	FoundConc.(µg/ml)
2.5	0.207	2.56
05	0.395	4.91
7.5	0.602	7.49
10	0.806	10.04
12.5	1.003	12.5

Table No.12 Results for linearity of UV Spectroscopic method

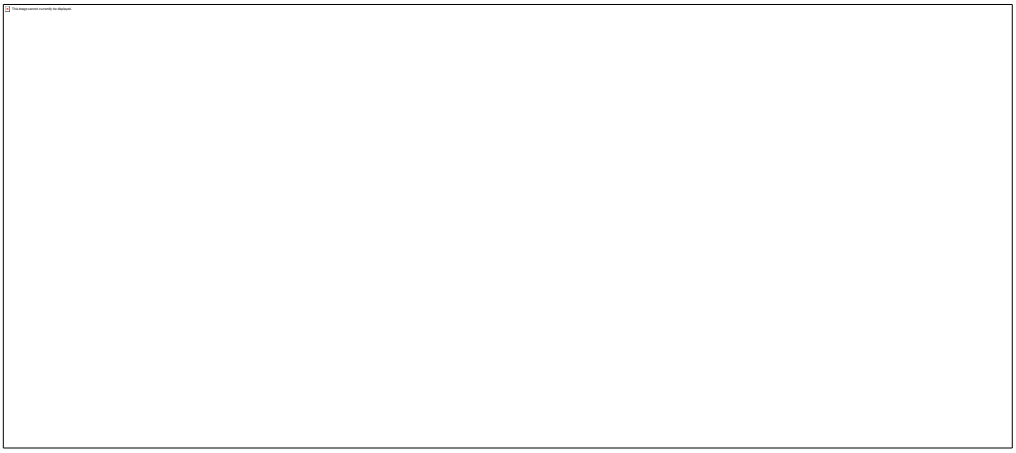


Fig No.1 Calibration curve of Cetilistat

Precision:

Intraday Precision:

1) Morning Precision:

Concentration(µg/ml)	Absorbance	Foundconc.	%F.C.	Mean	% RSD
2.5	0.203	2.51	100.4	0.205	1.22
2.5	0.206	2.55	102		
2.5	0.208	2.57	102.8		

5	0.397	4.93	98.6	0.399	1.09
5	0.396	4.92	98.4		
5	0.404	5.02	100.4		
7.5	0.603	7.50	100	0.6006	0.821
7.5	0.595	7.40	98.6		
7.5	0.604	7.51	100.1		

Table No.3 Results for morning precision of UV Spectroscopic method

2)Afternoon Precision:

Concentration(µg/ml)	Absorbance	Foundconc.	%F.C.	Mean	% RSD
2.5	0.199	2.46	98.4	0.203	1.86
2.5	0.206	2.55	102		
2.5	0.205	2.53	101.2		
5	0.396	4.92	98.4	0.399	1.04
5	0.398	4.94	98.8		
5	0.404	5.02	100.4		
7.5	0.601	7.48	99.7	0.600	0.75
7.5	0.605	7.53	100.4		
7.5	0.596	7.41	98.8		

Table No.4 Results for afternoon precision of UV Spectroscopic method

3) Evening Precision:

Concentration(µg/ml)	Absorbance	Foundconc.	%F.C.	Mean	% RSD
2.5	0.203	2.51	100.5	0.204	0.84
2.5	0.206	2.55	102		
2.5	0.203	2.51	100.5		

5	0.397	4.93	98.7	0.399	0.501
5	0.399	4.96	99.2		
5	0.401	4.98	99.7		
7.5	0.602	7.49	99.9	0.602	0.67
7.5	0.607	7.55	100.7		
7.5	0.599	7.45	99.4		

Table no.5 Results for evening precision of UV Spectroscopic method

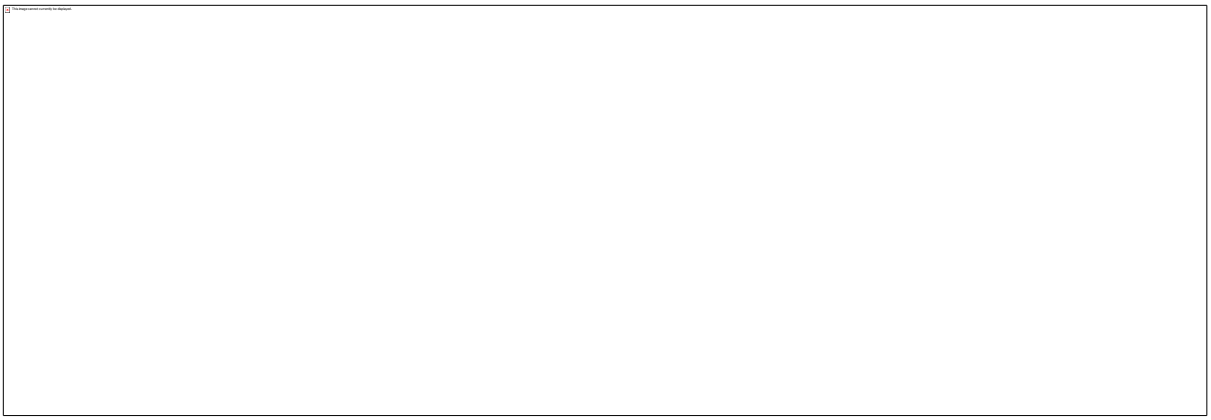
4)Interday Precision:

Concentration(µg/ml)	Absorbance	Foundconc.	%F.C.	Mean	% RSD
2.5	0.203	2.51	100.5	0.204	0.84
2.5	0.206	2.55	102		
2.5	0.203	2.51	100.5		
5	0.397	4.93	98.7	0.399	0.501
5	0.399	4.96	99.2		
5	0.401	4.98	99.7		
7.5	0.602	7.49	99.9	0.602	0.67
7.5	0.607	7.55	100.7		
7.5	0.599	7.45	99.4		

Table No.6 Results for interday precision of UV Spectroscopic method

Accuracy:

Table No.7Results for interday precision of UV Spectroscopic method



.4 LOD And LOQ:

Parameters	Result(µg/ml)
LOD	0.103
LOQ	0.314

Table No 7Results for LOD and LOQ of UV Spectroscopic method

9.6.5 Robustness:

Changein λmax(nm)	Concentration (ppm)	Absorbance	Found concentration	%F.C.	Mean	% RSD
215	5	0.394	4.89	97.8	0.3903	0.82
	5	0.389	4.83	96.6		
	5	0.388	4.82	96.4		
219	5	0.394	4.89	97.8	0.3906	0.64
	5	0.390	4.84	96.8		
	5	0.388	4.82	96.4		

Table no.7 Results for robustness of UV Spectroscopic method

9.6.6 Ruggedness:

Concentration(µg/ml)	Absorbance	Foundconc.	%F.C.	Mean	% RSD

2.5	0.210	2.6	104	0.211	1.25
2.5	0.214	2.65	106		
2.5	0.209	2.58	103.5		
5	0.419	5.2	104.1	0.418	0.27
5	0.417	5.18	103.6		
5	0.419	5.2	104.1		
7.5	0.603	7.5	100	0.599	0.60
7.5	0.596	7.41	98.9		
7.5	0.598	7.44	99.2		

Table No.8 Results for ruggedness of UV Spectroscopic method

9.7 Method Development and optimised chromatographic condition of HPLC

9.7.1 Selection of wavelength:

210 nm was selected for analysis of Cetilistat.

Fig no. 12 Selection of Wavelength

Fig. No.13 Chromatogram of trial 1

9.3 HPLC Method Validation:

9.3.1 Linearity:

Perform linearity at six levels over range of 25% to 150 % of working concentration. Prepared standard stock solution of Cetilistat and dilute suitably to obtain desired concentration at about 25%, 50%, 75%, 100%, 125% and 150%.

Result:

Sr. No.	Sample name	Mean Peak Area	Conc. (ppm)
1	Linearity 1 (25%)	445862	2.56
2	Linearity 2 (50%)	891725	5.12
3	Linearity 3 (75%)	1337587	7.68
4	Linearity 4 (100%)	1783450	10.24
5	Linearity 5 (125%)	2229321	12.89
6	Linearity 6 (150%)	2675175	15.36
Correlation Coefficient		1	
Slope		120007	
Y- Intercept		0.5333	
% Y- Intercept		1.45	

Table no.9 Results of linearity

Fig No. Linearity plot of Cetilistat

Conclusion:

It is concluded from the above observations that method is linear over range from 25 % to 150 % of specification level for Cetilistat.

Precision:

1. System Precision:

The HPLC system received five injections in replication. The peak response five replicate injections should have a % RSD less than or equal to 2.0.

Result:

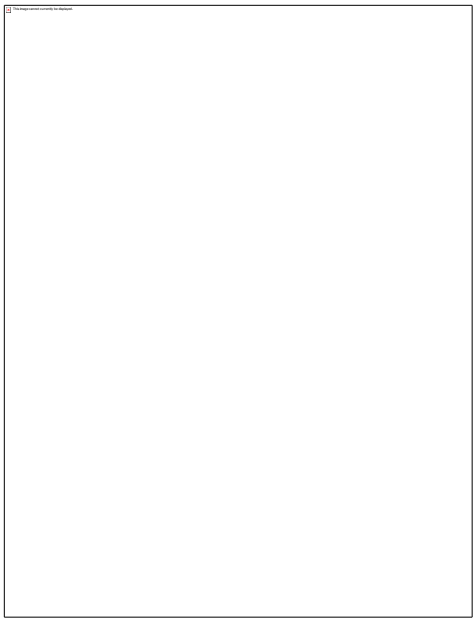


Table No.10 Results of system precision

Conclusion:

It is concluded from above results that the system complies with the acceptance criteria of system precision.

2. Method Precision:

In the precision method, the analyze the same batch sample six times perform for the consistency. This shows if a method is providing reliable results for one batch. The % the consistency. This shows if a method is providing The % RSD of the six measurements must not exceed 2.0

Sample no.	Sample in mg	Area			% Assay
		Injection 1	Injection 2	Average Area	
1	410.2	1747439	1747553	1747496	98.96

2	408.15	1744066	1745597	1744832	9.3
3	409.36	1795828	1796069	1795949	101.91
4	412.12	1774339	1771627	1772983	99.93
5	408.35	1780204	1774857	1777531	101.11
6	410.45	1773474	1782294	1777884	100.62
				Average	100.31
				SD	1.121
				% RSD	1.21

Table no.11 Results of method presicion

Conclusion:

% Assay obtained by six Assay samples preparation found within the acceptance limit. Hence the given method is precise and repeatable.

9.3.3 Accuracy:

Accuracy of an analytical procedure performed at 3 levels (50%, 100% and 150%)

level No.	Actual amount added in mg	Average Area	Amount found in mg	% Recovery	Average	SD	%RSD
50	49.98	881756	49.79	99.62	99.61	0.72	0.72
	50.11	890281	50.27	100.32			
	50.35	881811	49.79	98.89			
100	100.41	1782450	100.65	100.24	100.32	0.38	0.38
	99.81	1767217	99.79	99.98			
	100.24	1788125	100.97	100.73			
150	150.89	2664206	150.44	99.7	99.73	0.12	0.12
	150.62	2657498	150.06	99.63			
	150.96	2669936	150.76	99.87			

Table no12. Results for Accuracy

Specificity:

Reading are:

Sample no	Sample weigh	Area			% Assay
		Injection 1	Injectio n 2	Average Area	
1	410.2	1747439	1747553	1747496	98.96
2	408.15	1744066	1745597	1744832	99.3
3	409.36	1795828	1796069	1795949	101.91
				Average	100.06
				SD	1.614
				% RSD	1.61

9.3.5 Range:

Linearity at six levels over the range of 25% to 150% of the working concentration.

9.3.6 LOD and LOQ:

9.3.7 Robustness:

Readings are:

1) By applying plus temperature:

sample no.	Weight of sample in mg	Area			% Assay
		Injection1	Injection 2	Average area	
1	406.5	1761452	1761452	1762452	98.09
2	402.1	1764523	1764523	1766490	99.39

3	411.2	1776821	1776821	1774701	97.65

Table no.13 Results for plus temperature of robustness

2) By applying minus temperature:

sample no.	Weight of sample in mg	Area			% Assay
		Injection1	Injection 2	Average area	
1	406.5	1762451	1761987	1762219	100.38
2	402.1	1758681	1752146	1755414	101.09
3	411.2	1752648	1756753	1754701	98.81

Table no. 14 Results for minus tempetature of robustness

9.3.8 Ruggedness:

The ruggedness or Intermediate precision of a method was assessed to check the effect of change in analysis on analysis of Cetilistat.

sample no.	Weight of sample in mg	Area			% Assay
		Injection1	Injection 2	Average area	
1	406.5	1761452	1761452	1762452	98.09
2	402.1	1764523	1764523	1766490	99.39
3	411.2	1776821	1776821	1774701	97.65
4	412.02	1788112	1790513	1789313	98.15
5	410.02	1795120	1798987	1797054	99.06
6	410.36	1786120	1788512	1787316	98.44

Average	98.52
SD	0.414
%RSD	0.42

Table no.15 Results of Ruggedness

9.3.9 Repeatability:

Readings are:

sample no.	Weight of sample in mg	Area			% Assay
		Injection1	Injection 2	Average area	
1	406.5	1762451	1761987	1762219	100.38
2	402.1	1758681	1752146	1755414	101.09
3	411.2	1752648	1756753	1754701	98.81

Table no16. Results of Repeatability

9.3.10 Solution Stability:

Readings are:

1. **Standard Solution Stability:**

Time in hours	Peak area	% Assay	Difference
Initial	1763908	101.04	0
2	1770070	101.4	0.36
4	1762518	100.96	0.08
6	1762472	100.96	0.08
8	1766360	101.18	0.14
10	1768062	101.28	0.24
12	1765850	101.15	0.11
14	1766946	101.22	0.18

16	1769320	101.35	0.31
18	1771541	101.48	0.44
20	1768415	101.3	0.26
22	1770789	101.44	0.4
24	1752684	100.4	0.64
		0	101.04
		0	101.04
		0	101.04

Table no. 17Results of Standard Solution Stability

2. Sample Solution Stability

Time in hours	Peak area	% Assay	Difference
Initial	1747439	98.95	0
2	1750555	99.13	0.18
4	1749458	99.07	0.12
6	1736851	98.35	0.6
8	1748987	99.04	0.09
10	1738564	98.45	0.5
12	1745684	98.85	0.1
14	1742580	98.68	0.27
16	1756482	99.47	0.52
18	1747526	98.92	0.01
20	1752681	99.25	0.3
22	1746826	98.92	0.03
24	1752684	99.25	0.3
		0	98.95
		0	98.95
		0	98.95

Table no18. Results of Sample Solution Stability

Conclusion:

Therefore, Standard & sample solutions are stable up to 24 hours.

SUMMARY AND CONCLUSION

The anti-obesity medication Cetilistat was analyzed in tablet dose form using a straightforward, efficient and accurate HPLC method. Finding a safe and affordable platform was the primary goal of this research. It was revealed that cetilistat has a wavelength of 210 nm when evaluated in its dosage form frames using the RP-HPLC technique. A 150 mm × 4.6 mm Zodiac C18 column equipped with reversed-phase technology and kept at a temperature of 30°C is used for the elution procedure. For the isocratic analysis mode, the column was balanced using a mobile phase consisting of 50:50 ACN and Buffer pH 4.4. We detected with a Diode Array Detector at 210 nm at a flow rate of 1.0 mL/min.T here was a 3.477-minute retention time observed for the anti-obesity drug. Based on the accepted criteria of the established approach, the results of the system suitability test were satisfactory. A linearity range of 25% to 150% and an R2 value of 0.999 were used in the validation, which was done in conformity with the standards of ICH Q2 (R1). A hundred and six percent recovery rate was achieved with this medication. Both the limit of detection (LOD) and the limit of quantification (LOQ) were found to be 0.012µg/ml and 0.043µg/ml, respectively. With such a little percentage RSD, it seems like the method is spot on. The approach was shown to be drug-specific according to the findings of the specificity analysis. The proposed approach is now being used to validate the cetilistat analytical technique. Developing and validating HPLC techniques is the primary objective of this study, which intends to investigate and assess Cetilistat. Results from all techniques were in excellent agreement with each other and with the specified value of the pharmaceutical formulation; this was after validation according to ICH guidelines. There is a high degree of congruence between the results obtained using the suggested approach.Furthermore, considering the ongoing evaluation of Cetilistat, the HPLC method's ongoing development can be perceived as trustworthy, sensitive, accurate, transparent, and precise.

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