

PRODUCT DEVELOPMENT, CHARACTERIZATION AND SPECTROPHOTOMETRIC EVALUATION OF CETILISTAT SELFMICROEMULSIFYING DRUG DELIVERY SYSTEM

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ABSTRACT

BCS-Class-IV drugs such as Cetilistat have challenges like developing a product with desirable oral bioavailability and method estimation by routine analysis. In present, self-microemulsion drug delivery system was formulated to develop drug in more soluble and resolve the analytical estimation issues and check the feasibility of UV Spectrophotometric method. Solubility of Cetilistat was determined in various oils, surfactants, and co-surfactants. Pseudo ternary phase diagrams were constructed to identify the microemulsion region. From pseudo-ternary diagram, different S_{mix} ratios were selected to identify optimum formulation. The formulation was evaluated for identification test, droplet size, zeta potential, and drug content. The optimum formulation was 12.5% of oil (IPM), 50% of Surfactant and co-surfactant (Milcoside-200 and Acconon MC8). This formulation was converted to solid SMEDDS using adsorbent like Aerosil. The method estimation of prepared liquid formulation was done by UV Spectrophotometric method. The method was found simple,

accurate, precise and effective in the routine analysis without any interference of excipients used in SMEDDS formulation.

KEYWORDS: BCS Class-IV, Cetilistat, SMEDDS Formulation, Method Estimation.

1. INTRODUCTION

Cetilistat is a drug designed to treat obesity. Cetilistat is a novel synthetic, highly lipophilic benzoxazinone derivative that acts as a potent inhibitor of pancreatic lipase. The drug inhibits the activity of lipase, a lipolytic enzyme, secreted by the digestive tract and pancreas, and blocks the absorption of fat from the gut, resulting in reduced body weight as well as for the treatment of type-II diabetes.^[1] Cetilistat has completed Phase I, II & III trials in Japan.^[2] Cetilistat is a BCS Class -IV drug with Poor Bioavailability & Poor Solubility.

Micro-emulsion technologies, particularly SMEDDS (Self Micro-Emulsifying Drug Delivery System), offer a promising approach to overcome solubility limitations through particle size reduction, increased surface area. These systems are essentially mixing of oil and surfactant (sometimes with added co surfactant) that form emulsion on mixing with water with little or no energy input. These fine O/W emulsions produce small droplets of oil dispersed in the gastro-intestinal fluids that provide a large interfacial area increasing the activity of pancreatic lipase to hydrolyse triglycerides and thereby, promote a faster release of drug or formation of mixed micelles of the bile salts containing drug.^[3] SMEDDS are physically stable & transparent formulations with droplet size less than 50 nm that are easy to manufacture. Thus, for lipophilic drug compounds that exhibit dissolution rate-limited absorption, these systems may offer an improvement in the rate and extent of absorption and result in more reproducible blood-time profiles.^[4] The Conversion of liquid SMEDDS into Solid SMEDDS is a common Pharmaceutical approach to improve stability, handling & Patient Compliance while retaining the therapeutic benefits of liquid formulations. The present study is designed to formulate Solid SMEDDS using suitable adsorbent carriers,^[5] & Perform Characterization with Spectrophotometric Evaluation.

2. MATERIALS & METHODS

Cetilistat was Procured from Intas Pharmaceuticals, Ahmedabad. Other chemicals & reagents used were analytical grade.

2.1 Estimation of Cetilistat

2.1.1 Preparation of Standard Curve of Cetilistat

Cetilistat was accurately weighed (2.5 mg) and transfer into 25 ml of volumetric flask. Acetonitrile was added to dissolve and prepare standard stock solution (SS) of 100 μ g/ml. From Standard stock solution (SS) aliquots of 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml, and 0.6 ml were pipette out into 10 ml volumetric flasks. The volume was made up with Acetonitrile

to get final concentration of 1, 2, 3, 4, 5, 6 $\mu\text{g}/\text{ml}$, respectively. The absorbance of each concentration was analysed at λ_{max} of 227 nm.

2.2 Construction of Pseudoternary Phase Diagrams^[6,7,8]

Surfactant was blended with co-surfactant in the ratio of 1:1. This Surfactant: Co-surfactant (S_{mix}) ratios were chosen for detailed study of phase diagrams. Aqueous titration method was used for the construction of Pseudoternary phase diagram. Different combinations in different weight ratios of oil and S_{mix} , 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 were taken.

2.3 Preparation of Liquid SMEDDS

Liquid SMEDDS formulation was prepared by dissolving 10 mg of Cetilistat in the optimized SMEDDS mixture consisting of Isopropyl myristate, Milcoside 200, Acconon MC8-2. In this mixture water was added dropwise with Continuous stirring. This mixture was observed for any signs of turbidity or phase separation for a period of 48 hours.

2.3.1 Characterization of Liquid SMEDDS.^[9,10]

2.3.1.1 Visual Inspection

These systems were visually inspected for homogeneity, optical clarity, and fluidity.

2.3.1.2 Dye Solubility Test

Add 2-3 drops of water-soluble dye such as crystal violet in the microemulsion formulation. The type of emulsion observed after 5 minutes.

2.3.1.3 Particle Size & Zeta Potential Analysis

The particle size of resultant liquid SMEDDS were measured using Dynamic Light Scattering. The liquid SMEDDS samples were taken in disposable glass tubes and particle size was determined.

2.3.1.4. Freeze Thaw Stability Study

Three freeze thaw cycles between -21°C and 25°C, with formulation storage at each temperature for not less than 48 hours were performed & microemulsion formulation was observed for instability.

2.3.1.5 Drug Content

The drug content of the microemulsion formulation was determined by dissolving 1 ml of formulation in 10 ml of Acetonitrile. Stir it for 30 minutes using magnetic stirrer. After

stirring centrifuge, it for 10 minutes. After suitable dilutions with acetonitrile, absorbance was determined using the UV spectrophotometer at wavelength 227 nm.

2.4 Preparation of Solid SMEDDS (S-SMEDDS)^[11]

The optimized liquid SMEDDS formulation was converted into free-flowing powder by adsorption of liquid SMEDDS on to solid carriers. The solid carrier used includes colloidal silicon dioxide (Aerosil). Sufficient amount of aerosil was used to prepare solid SMEDDS.

2.4.1 Characterization of SOLID SMEDDS^[12,13]

2.4.1.1 Angle of Repose

A funnel was kept vertically in stand at a specified height above a paper placed on horizontal surface. The bottom was closed and 2 gm of sample powder was filled in funnel. The funnel was opened to release the powder on paper to form a smooth conical heap. The height of heap was measured using the scale. The angle of repose was calculated using following formula:

$$\theta = \tan^{-1} \left(\frac{h}{r} \right)$$

Where, h= height of the heap & r = radius of the heap

2.4.1.2 Bulk Density

The bulk density depends on density of powder particles and the arrangement of the powder particles. The bulk density is obtained by adding a known mass of powder to a graduated cylinder. The density is calculated as mass/volume (g/cc).

2.4.1.3 Tapped Density

The tapped density is an increased bulk density attained after mechanically tapping a container containing the powder sample. The mechanically tapping is achieved by raising the cylinder or vessel and allows dropping, under its own mass, from a specified distance. The density is calculated as mass/volume (g/cc).

2.4.1.4 % Porosity

Porosity or void fraction is a measure of void spaces in a material, and is a fraction of voids over the total volume.

$$\% \text{ Porosity} = \left(\frac{\text{Volume of voids}}{\text{Total volume}} \right) \times 100$$

2.4.1.5 Compressibility Index and Hausner's Ratio

The compressibility index (Carr's Index) and Hausner's ratio are measures of the products ability to settle, and permit an assessment of relative importance of interparticulate interactions.

$$\text{Compressibility index} = \left(\frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \right) \times 100$$

$$\text{Hausner's ratio} = \left(\frac{\text{Tapped density}}{\text{Bulk density}} \right)$$

2.5 Validation & Estimation of Cetilistat in Formulation^[14,15]

2.5.1 Linearity (Calibration curve)

The Calibration curves were plotted over a concentration range of 1-6 µg/ml for Cetilistat using Acetonitrile. The absorbance of solution was measured at 227 nm. The calibration curves were constructed by plotting absorbance versus concentration and the regression equation was calculated.

2.5.2 Repeatability

The precision of instrument was checked by repeated scanning and measurement of absorbance of solution (n=6) for cetilistat (1-6 µg/ml) without changing the parameter of the Spectrophotometric method. The results are reported in terms of relative standard deviation (%RSD).

2.5.3 System Precision

2.5.3.1 Intraday Precision & Interday Precision

Test solutions containing 2, 4, and 6µg/ml were analysed three times on the same day & three different days for Intraday & Interday precision respectively. The absorbance was measured at 227 nm.

2.5.4 Sensitivity

2.5.4.1 Limit of Detection and Limit of Quantification (LOD & LOQ)

The limit of detection and limit of quantification were calculated using the following equation:

$$\text{LOD} = 3.3 \frac{\sigma}{s}$$

$$\text{LOQ} = 10 \frac{\sigma}{s}$$

Where, σ = Standard deviation of y intercept of calibration curve ($n = 6$)

S = Slope of regression equation

2.5.5 Accuracy

The accuracy of the method was determined by calculating recovery of cetilistat in formulation by standard addition method. Known amounts of standard solutions of cetilistat were added at 50, 100, and 150 % level to pre-quantified sample solutions of cetilistat (2 μ g/ml). The amounts of cetilistat were calculated by applying the regression line equations. The experiment was repeated for three times.

2.5.6 Robustness

Robustness was studied by analyzing the samples of Cetilistat by deliberate variation in the method parameters. The change in the results of Cetilistat was noted. Robustness of the method was studied by changing the wavelength ± 1 nm.

3 RESULTS AND DISCUSSION

3.1 Standard Curve of Cetilistat

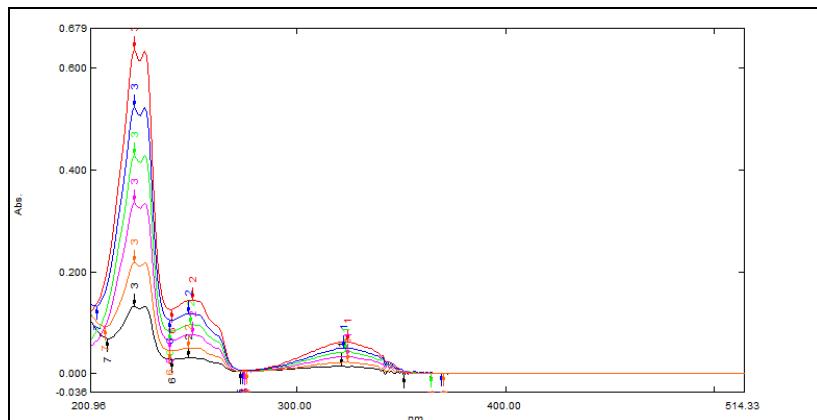


Fig. 1: Determination of Absorbance of Different Concentration of Cetilistat at 227 nm λ_{max} .

Table 1: Absorbance of Cetilistat in Acetonitrile.

Concentration (μ g/ml)	Absorbance			Mean \pm SD
1	0.118	0.113	0.117	0.116 \pm 0.0020
2	0.230	0.226	0.225	0.227 \pm 0.0020
3	0.313	0.311	0.312	0.312 \pm 0.0010
4	0.405	0.404	0.404	0.404 \pm 0.0005
5	0.508	0.513	0.515	0.512 \pm 0.0036
6	0.604	0.613	0.621	0.612 \pm 0.0085

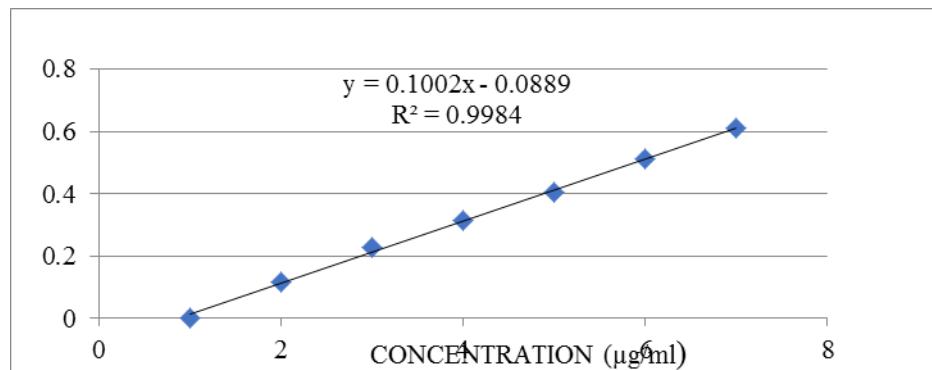


Fig. 2: Calibration Curve of Cetilistat.

3.2 Pseudo-Ternary Phase Diagram

Solubility of drug in mixture of Isopropyl myristate, Milcoside-200, and Acconon MC8 were selected for the construction of pseudoternary phase diagram. Total nine combinations were prepared. 1:1 ratio was best for the formulation due highest entrapment efficiency of water phase and transparency also there after 24 hours.

Table 2: Compositions of SMEDDS.

Drug (mg)	S_{mix}	% Oil phase	% S_{mix}	% water phase
10	1:1	0.09	0.89	99.98
		0.19	0.79	99.00
		23.00	53.84	23.07
		33.33	50.00	16.66
		45.45	45.45	9.09
		54.54	36.36	9.09
		63.63	27.27	9.09
		72.20	18.10	9.09
		81.80	9.09	9.09

*All ingredients represent in % v/v.

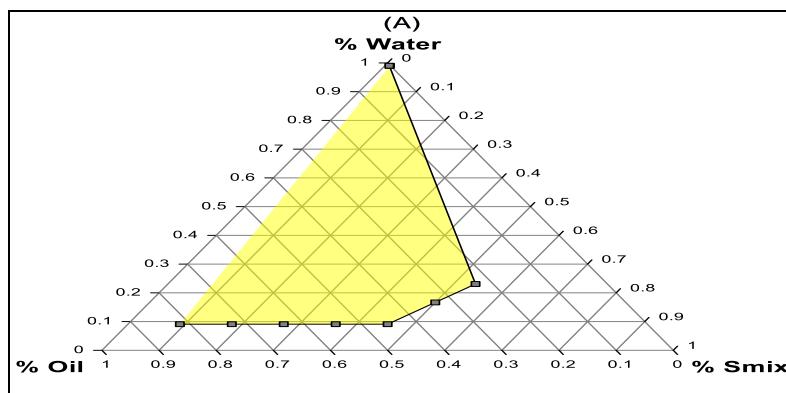


Fig. 3: Pseudoternary Phase Diagram of Microemulsion Containing Oil (IPM), Surfactant (Milcoside 200), Co-Surfactant (Acconon Mc8) with S_{mix} Ratio 1:1,

Table 3: Composition of Liquid SMEDDS.

S _{mix} Ratio	Drug(mg)	Oil%	Surfactant: Co-surfactant %	Water%
1:1	10	12.5	50	37.5

¹Oil phase: Isopropyl myristate (IPM)

²Surfactant: Milcoside-200

³Co-surfactant: Acconon MC8

⁴Drug: Cetilistat

**Fig. 4: SMEDDS Formulation of Cetilistat.**

3.3 Characterization of Liquid SMEDDS

3.3.1 Identification Test/ Dye Solubility Test

In liquid SMEDDS, Water soluble dye (Crystal violet) added. In formulation there is uniform distribution of dye and forms clear, transparent microemulsion. The optimized liquid SMEDDS forms O/W type of emulsion.

**Fig. 5: Dye Solubility Test.**

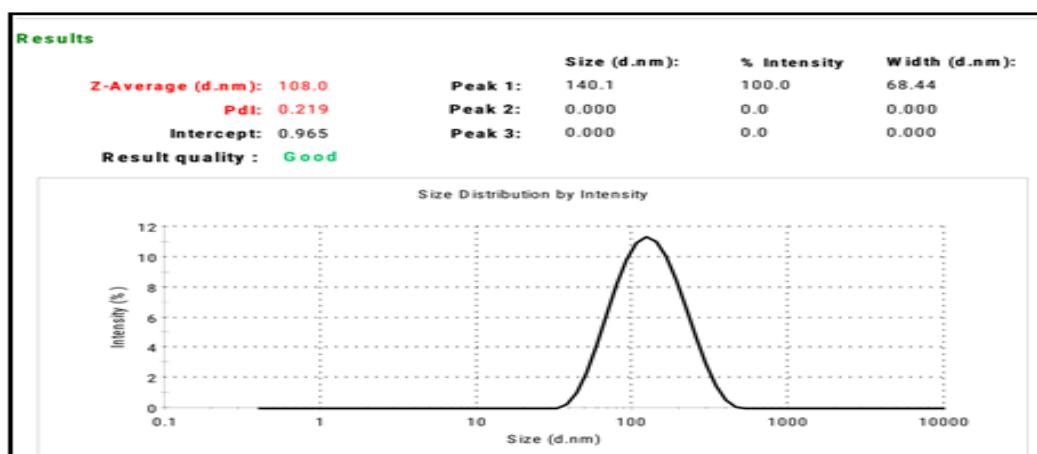
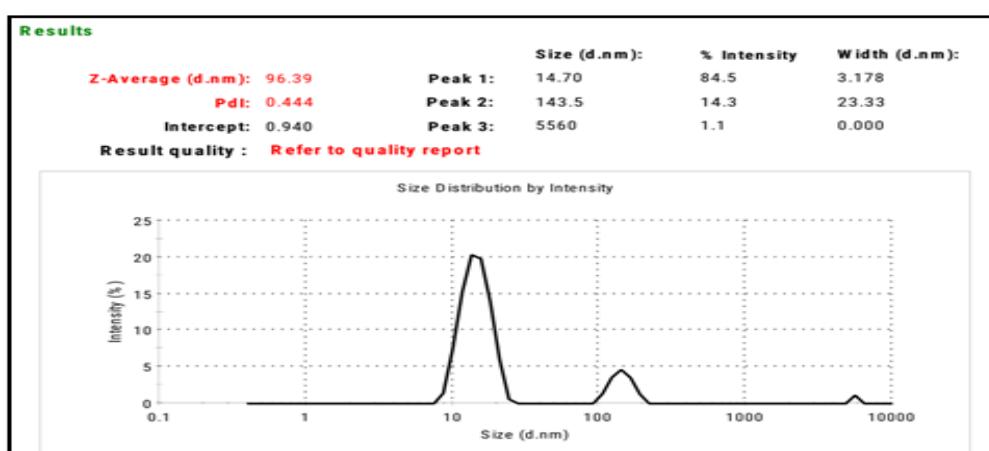
3.3.2 Particle Size Analysis

Particle size determines the emulsion stability thus affects the product shelf life.

Table 4: Particle Size Analysis of Microemulsion.

S _{mix} Ratio	Formulation	Size(nm)	PDI	% Intensity
1:1	Microemulsion without dilution	96.39	0.444	84.50
	Microemulsion prepared by dilution up to 25 ml water	108.00	0.219	100.00
	Microemulsion prepared by dilution up to 250 ml water	371.20	0.395	73.40

The particle size was performed using zeta-sizer by DLS. In this microemulsion were made and in that due to Brownian moment particle movement was analysed. It was observed that microemulsion prepared without dilution and microemulsion prepared with dilution up to 25 ml having lowest particle size, high intensity and having high stability than the microemulsion prepared by dilution up to 250 ml water.

**Fig. 6: Droplet Size Distribution of Microemulsion.****Fig. 7: Droplet Size Distribution of Microemulsion Diluted Up to 25 ml Water.**

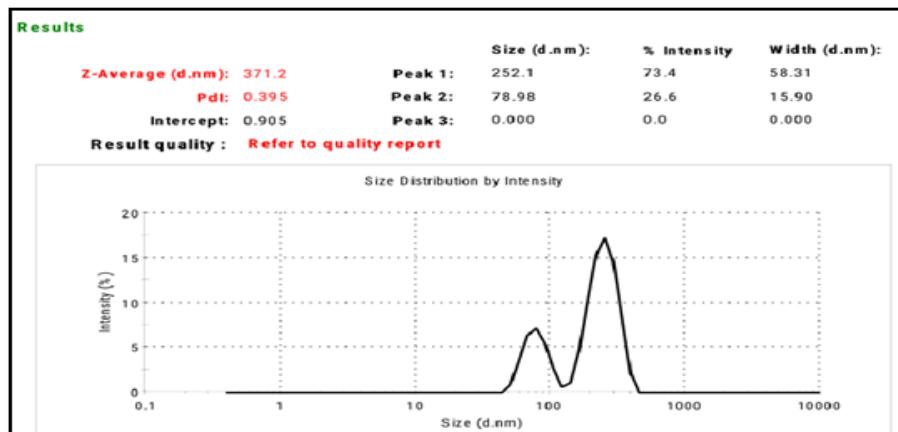


Fig. 8: Droplet Size Distribution of Microemulsion Diluted Up to 250 ml Water.

3.3.3 Zeta Potential Measurement

The significance of zeta potential is that its value can be related to short term or long-term stability of emulsions. In general, when the zeta potential of an emulsion is high, the repulsive forces exceed the attractive forces, resulting in a stable system.

Table 5: Zeta Potential Measurement of Microemulsion.

S _{mix} Ratio	Formulation	Zeta Potential(mV)	Area (%)
1:1	Microemulsion without dilution	-0.35	53.4
	Microemulsion prepared by dilution up to 25 ml water	-32.30	88.2
	Microemulsion prepared by dilution up to 250 ml water	-10.00	99.3

In zeta potential study, Zeta potential of microemulsion diluted up to 25 ml of water has the maximum value of potential.

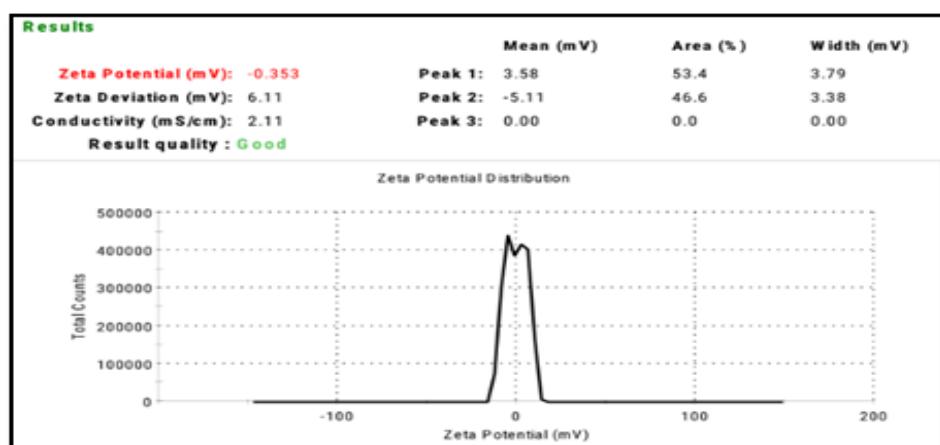


Fig. 9: Zeta Potential Distribution of Microemulsion

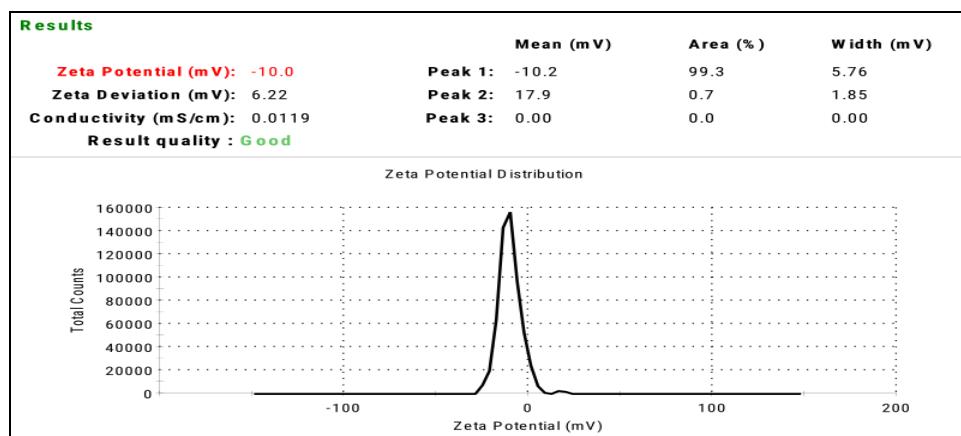


Fig. 10: Zeta Potential Distribution of Microemulsion Diluted Up to 25 ml Water

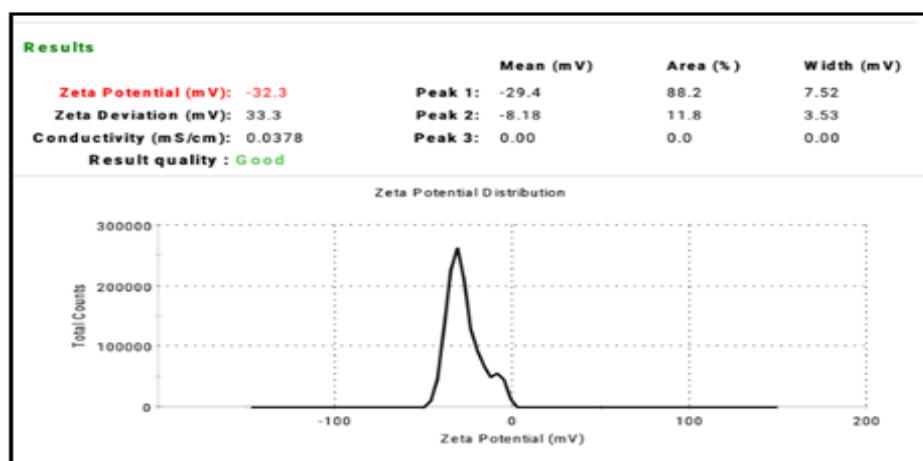


Fig. 11: Zeta Potential Distribution of Microemulsion Diluted Up to 250 ml Water

3.3.4 Freeze Thaw Stability Study

Stability study ensuring the maintenance of product quality, safety and efficacy throughout the shelf-life is considered for the acceptance and approval of any pharmaceutical product.

Table 6: Result of Freeze Thaw Stability Study.

Conditions	Particle size d. nm (PDI)	Zeta potential (mV)
At 25°C	362.5(0.671)	-0.417
At -21°C.	632.2(0.715)	-0.274

After performing the Freeze thaw stability study as shown in table 6, it was concluded that, the microemulsion formulation is stable only at room temperature.

3.3.5 Content of Cetilistat

Microemulsion was assayed spectrophotometrically for the drug content at the wavelength 227 nm with proper dilution of formulation taking Acetonitrile as blank.

Table 7: % Drug Content of Optimized Microemulsion (S_{mix}1:1)

Conc.(μ g/ml)	5	5	5	Mean	SD	% RSD
Drug content (%)	98.70	102.50	100.15	100.45	1.91	1.90

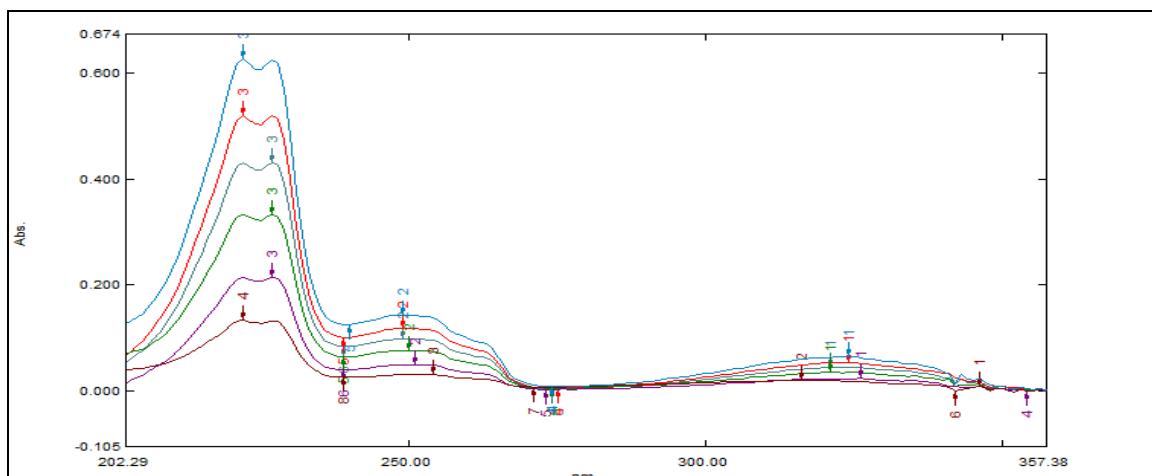
3.3.6 Characterization of Solid SMEDDS

Table 8: Flow Characteristics of Solid SMEDDS.

Parameters	Results	Flow property
Angle of repose(θ)	26.5°	Excellent flow property
Bulk density (g/cc)	0.15	-
Tapped density (g/cc)	0.20	-
Porosity (%)	24.0	-
Carr's Index	23.1	Passable flow property
Hausner's Ratio	1.30	Passable flow property

3.4 Validation and Estimation of Cetilistat in Formulation

3.4.1 Linearity (Calibration Curve)

**Fig. 12: Overlain Spectra of Cetilistat at 227 nm.**

The standard solutions for linearity were prepared 6 times at different concentration levels. Linear correlation was obtained between absorbance versus concentration at 227 nm.

Table 9: Regression Analysis Data.

Validation Parameters	Results
Analytical Wavelength (nm)	227
Linearity and range (μ g/ml)	1-6
Regression equation	$y=0.098x+0.023$
Correlation coefficient (R^2)	0.999
Limit of Detection (LOD) (μ g/ml)	0.175 μ g/ml
Limit of Quantification (LOQ) (μ g/ml)	0.53 μ g/ml

3.4.2 Repeatability

Repeatability of measurements of absorbance was evaluated using 6 replicates of same concentration (5 μ g/ml). The lower value of % RSD indicates that proposed method was found to be repeatable.

Table 10: Repeatability Data.

Sr.No.	1	2	3	4	5	6	Mean	SD	% RSD
Absorbance at 227 nm for Cetilistat (5 μ g/ml)	0.519	0.523	0.526	0.508	0.513	0.515	0.517	0.006653	1.2

3.4.2.1 Intraday and Interday Precision

The intraday and interday variation for the determination of Cetilistat was evaluated at different concentration levels. The low % RSD values of within a day and between the days for Cetilistat showed that proposed method was found to be precise.

Table 11: Precision Data (n=3).

Sample No.	Precision Data					
	Intraday			Interday		
Conc.(μ g/ml)	2 (μ g/ml)	4 (μ g/ml)	6 (μ g/ml)	2 (μ g/ml)	4 (μ g/ml)	6 (μ g/ml)
1	0.214	0.431	0.626	0.230	0.405	0.604
2	0.218	0.428	0.632	0.226	0.404	0.613
3	0.215	0.430	0.620	0.225	0.404	0.621
Mean	0.215	0.429	0.626	0.227	0.404	0.612
SD	0.0020	0.0015	0.0060	0.0026	0.0005	0.0085
% RSD	0.968	0.354	0.958	1.16	0.14	1.38

3.4.3 Accuracy

The samples of Cetilistat formulation were spiked with 50, 100, 150 % of standard Cetilistat and the mixtures were analysed by proposed method.

Table 12: Recovery Data (n=3).

Conc. Level %	Amount taken (μ g/ml)	Amount added (μ g/ml)	Amount Recovery (μ g/ml)	% Recovery \pm SD	% RSD
0	2	0	1.91	96.25 \pm 1.28	1.32
50	2	1	2.85	95.08 \pm 1.34	1.40
100	2	2	4.17	104.44 \pm 1.00	0.95
150	2	3	5.18	103.75 \pm 1.42	1.36

3.4.4 Robustness

Robustness of method was determined by measuring absorbance at $\lambda_{\text{max}} \pm 1$ nm of Cetilistat (227 nm). The low value of % RSD indicates the method is robust for given condition. Results are shown in table 13.

Table 13: Robustness Data (n=3).

A _{max}	Conc. (μg/ml)	Absorbance	Conc. recovered	% Recovery
226	5	0.489	4.75	95.00
227	5	0.496	4.82	96.53
228	5	0.502	4.88	97.75
Mean		0.495	4.81	96.42
SD		0.006506	0.06506	1.37
% RSD		1.31	1.35	1.42

4. CONCLUSION

In this study, self-micro emulsifying mixture containing oil, surfactant, and co-surfactant were prepared and their tendency to emulsify efficiently was evaluated. Excipients evaluated for self-micro emulsifying mixture were Tween80, Carbitol, Oleic acid, Olive oil, Isopropyl myristate, Propylene Glycol, Water+Methanol. All the excipients showed a tendency to form a microemulsion with varying degree of efficiency. A solid micro emulsifying mixture of Isopropyl myristate, Milcoside-200, Acconon MC8 was selected and optimized for delivering BCS Class IV drug to more solubilized form. Formulations with two Smix ratios were prepared. Among these formulations, of liquid SMEDDS, Formulation containing 12.5% Oil, 50% of surfactant and co-surfactant mixture was selected as optimized formulation. The formulation was characterized for different evaluation parameters like particle size measurement & Zeta potential measurement. Results of optimized liquid formulation indicated there was not any interaction of drug with excipients.

This optimized liquid formulation was converted in to solid SMEDDS. Solid SMEDDS was prepared using adsorption of liquid formulation on to the carrier and coating material. Aerosil was used as a solid carrier for conversion of liquid formulation to solid SMEDDS. Solid SMEDDS was evaluated for the flow property.

The UV Spectrophotometric method was estimated and validated using cetilistat and liquid SMEDDS preparation. The proposed UV Spectrophotometric method was found to be simple, sensitive, accurate, precise and robust. Hence the method was found economic for the estimation of cetilistat from the liquid SMEDDS preparation. The excipients which are

present in the Liquid formulation did not interfere in the analysis of cetilistat. The method was found effective in the routine analysis.

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